



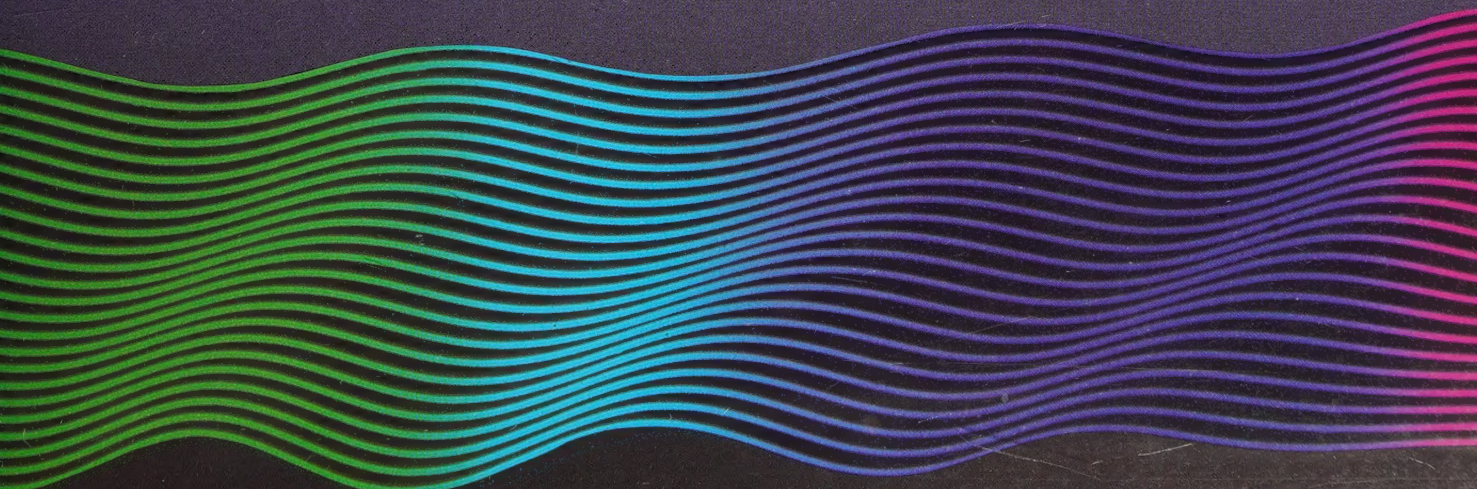
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Bioenergetics and PCB, DDE, and Mercury Dynamics in Lake Ontario Lake Trout (*Salvelinus namaycush*): A Model based on Surveillance Data

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Borgmann, U., and D. M. Whittle. 1992. Bioenergetics and PCB, DDE, and mercury dynamics in Lake Ontario lake trout (*Salvelinus namaycush*): a model based on surveillance data. *Can. J. Fish. Aquat. Sci.* 49: 1086–1096.

Lake trout (*Salvelinus namaycush*) ingestion rates in a bioenergetics and contaminant dynamics model were estimated directly from contaminant concentrations in lake trout and their prey, rather than from the sum of growth and predicted metabolism. Elimination rates for PCB and DDE, but not for mercury, were dependent on either body mass or lipid content. Concentrations in lake trout responded rapidly to changes in concentration of their prey. This was due primarily to growth dilution and not contaminant elimination, especially for DDE and PCB. Changes in lipid concentrations, therefore, have only minor effects on final concentrations in lake trout, and it is not appropriate to lipid normalize PCB or DDE concentrations when examining trends in whole-body concentrations for this species. Concentrations of PCBs and lipids have declined in lake trout from 1977 to 1988. The drop in PCB concentrations is probably not caused primarily by the lowered lipid concentrations but is the result of either a reduction in feeding rates and improved growth efficiencies, a reduction in PCB concentrations in alewife (*Alosa pseudoharengus*), or an undocumented change in prey selection. Models based on chemical kinetics across the gastrointestinal tract are more consistent with observed data than models based on a constant contaminant assimilation rate and direct excretion.

On a estimé le taux d'ingestion des contaminants chez le touladi (*Salvelinus namaycush*) d'après un modèle de bioénergétique et de dynamique des contaminants, en prenant comme point de départ la teneur en contaminants chez cette espèce et ses proies, plutôt qu'en se fondant sur les valeurs calculées selon la croissance et le métabolisme. Le taux d'élimination des PCB et des DDE, contrairement à celui du mercure, était tributaire de la masse corporelle ou de la teneur en lipides. Des variations de la concentration chez les proies ont provoqué une modification rapide de la concentration chez le touladi. On attribue cette variation principalement à la dilution résultant de l'accroissement de la masse corporelle, plutôt qu'à l'élimination des contaminants, surtout dans le cas des DDE et des PCB. Ainsi, des modifications dans la concentration des lipides n'ont que peu de répercussions sur les concentrations finales chez le touladi; donc il n'est pas indiqué de normaliser les concentrations en PCB ou en DDE par rapport aux lipides lorsque l'on étudie les tendances des concentrations dans l'ensemble de la masse corporelle chez les poissons de cette espèce. Chez le touladi, la teneur en PCB et en lipides a diminué entre les années 1977 et 1988. La chute de la concentration en PCB n'est probablement pas liée directement à la diminution de concentration en lipides, mais elle est plutôt imputable soit à une diminution des taux d'alimentation et à un meilleur rendement de croissance, soit à une baisse des concentrations en PCB chez le gaspareau (*Alosa pseudoharengus*), soit à un changement sans appui documentaire dans la sélection des proies. Les modèles axés sur la cinétique chimique le long du tractus gastro-intestinal concordent davantage avec les données recueillies que les modèles reposant sur un taux constant d'assimilation des contaminants et d'excrétion directe.

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Concentrations of PCB, DDE, and mercury in lake trout (*Salvelinus namaycush*) and two forage species, rainbow smelt (*Osmerus mordax*) and slimy sculpin (*Cottus cognatus*), in Lake Ontario have been measured since 1977 by the Department of Fisheries and Oceans as part of an international surveillance program (Borgmann and Whittle 1991, 1992). In addition to their importance as a monitoring tool, these data provide some valuable insights into trophic relationships and raise serious questions about how such data should be interpreted. For example, PCB concentrations in lake trout show strong oscillations superimposed on a gradual decline. The oscillations correlate with changes in the growth rate of the alewife (*Alosa pseudoharengus*) population, an important forage fish, suggesting that PCB biomagnification in Lake Ontario

is strongly influenced by food web interactions (Borgmann and Whittle 1991). In sharp contrast with data obtained from lake trout, PCB concentrations in rainbow smelt and slimy sculpin did not decline appreciably from 1977 to 1988. The ratio of PCB concentrations in lake trout to those in rainbow smelt has declined, roughly in parallel to declines in lipid concentrations in lake trout (Borgmann and Whittle 1992). Is it possible that PCB biomagnification is a function of lipid concentrations in lake trout? Alternatively, is it possible that both lipid concentrations and the biomagnification factor have changed as a result of changes in feeding rates and biomass conversion efficiencies? This is an important question because PCB and DDT are concentrated in lipids, and some authors recommend normalizing PCB and DDT concentrations to lipid (e.g. Hamelink

et al. 1971; Thomann 1989). Uptake and excretion of contaminants can, at least in some situations, be directly related to the adiposity of fish tissues (Roberts et al. 1977).

There are other questions raised by the contaminant surveillance data. The oscillations in PCB concentrations in lake trout are relatively uniform among all age classes (Borgmann and Whittle 1991). There was no detectable lag in the older fish relative to the younger fish, as might be expected if PCB concentrations are the result of a lifetime of accumulation and retention. This suggests that concentrations of these strongly biomagnified compounds, which are excreted very slowly (Niimi and Oliver 1983), nevertheless respond rapidly to changes in concentrations in prey. Is this possible in a long-lived species such as the lake trout? Furthermore, what is the impact of prey selection on concentrations in lake trout? There have been major changes in the forage fish stocks in Lake Michigan in recent years (Jude and Tesar 1985). What effect might future changes in Lake Ontario forage fish populations have on contaminant concentrations in predators?

The above questions cannot be investigated from examination of the surveillance data alone. A deeper understanding of the bioenergetics and contaminant uptake and elimination rates is required. Therefore, we have used the contaminant surveillance data to develop relatively simple models of lake trout bioenergetics and PCB, DDE, and mercury dynamics. Growth rate data were available from the surveillance data, and prey ingestion rates were estimated from predator and prey contaminant concentrations using procedures similar to Niimi (1981). For simplicity, contaminant uptake from water was ignored. Several studies suggest that most of the persistent contaminants accumulated in salmonids are obtained from food, and not from water (Spigarelli et al. 1983; Thomann and Connolly 1984; Oliver and Niimi 1988; Rasmussen et al. 1990). Contaminant assimilation and elimination rates were modelled using two different approaches: a model of chemical exchange between the gastrointestinal tract and the rest of the fish, or a model using constant assimilation rate and an excretion rate dependent on body size or lipid content.

Theory

The equation for biomass conversion can be written

$$I = A + F = G + F + M$$

where I is the food ingested, A is assimilation, F is egestion, G is growth, and M is metabolism and excretion. The terms of greatest interest with respect to feeding and biomass conversion efficiencies are I and G . G can be estimated directly from the weight of lake trout at different ages. I can be estimated from contaminant bioaccumulation. Two different models were used to do this.

Model 1

In Model 1, contaminant uptake from, and excretion into, the water are ignored. Contaminant uptake and loss are assumed to be entirely controlled by contaminant flow across the gastrointestinal lining. The rate of change in the body burden of contaminant (B) is then given by

$$(1) \quad \begin{aligned} dB/dt &= E \cdot I \cdot C_i \\ &= E_0 \cdot I \cdot C_i - k_e \cdot B \end{aligned}$$

where E is net contaminant assimilation efficiency, C_i is the contaminant concentration in the ingested food, E_0 is the max-

imum contaminant assimilation efficiency obtained when the body burden is 0, and k_e is the apparent contaminant elimination rate (per day). If the body burden is 0, then k_e is also 0 and $E = E_0$. The contaminant elimination rate for equation 1 can be described by (see Appendix)

$$(2) \quad k_e = E_0 \cdot K_{eq} \cdot f \cdot (I/W)$$

where K_{eq} is the chemical equilibrium between the contaminant in the body of the fish and the gut, $f = (I - A)/I$, and W is the fish's body mass.

Model 2

A more conventional model, ignoring the dynamics of digestion, is

$$(3) \quad dB/dt = E_0 \cdot I \cdot C_i - k_e \cdot B$$

where

$$(4) \quad k_e = a \cdot W^{-n}$$

Contaminant elimination rate is assumed to be only a function of body size in Model 2. A special case, where k_e is constant, occurs if $n = 0$.

In Model 2 the assimilation efficiency (E_0) is assumed to be constant and independent of concentration in the body. This obviously cannot be entirely correct, and Model 1 is thermodynamically more reasonable. However, models equivalent to Model 2 have been used more frequently in contaminant modelling (e.g. Norstrom et al. 1976; Thomann and Connolly 1984). Furthermore, Model 1 does not take into account direct excretion of contaminant into the water through the gills or urine, or contaminant metabolism. Model 1 predicts that the apparent contaminant elimination rate (k_e) is a function of ingestion rate whereas Model 2 does not. Model 1 is a little simpler to fit to experimental data, since only a single constant needs to be estimated in k_e (the product of K_{eq} and f) whereas two constants need to be estimated in Model 2 (a and n). A combination of Models 1 and 2 would probably be best, if contaminant elimination through faeces and other pathways could be distinguished. Since we had insufficient data to do this, both models were used and the results compared.

Parameter Estimation

Lake Trout Growth Rate

The equation chosen to describe the instantaneous rate of growth (g , per year) for lake trout was

$$(5) \quad g = g_m / (1 + uW^v)$$

When fitted by least squares to the lake trout data for body weight at ages 1–8 (Fig. 1), using a computer model with daily iterations in body weight, this gave estimates of $g_m = 1.7/\text{yr}$, $u = 4.1 \times 10^{-5}$, and $v = 1.5$ at an initial weight of 68 g ($R^2 = 0.998$ based on log W).

Prey Species and Size Selection

Slimy sculpin make up the largest portion of the diet of young lake trout (Elrod 1983; Christie et al. 1987). Large fish feed predominantly on alewife and rainbow smelt. Alewife are usually reported to be the dominant diet (Brandt 1986; Christie et al. 1987), but rainbow smelt may become more important during the summer months (Elrod 1983; Olson et al. 1988) when lake trout are separated from alewife by the thermocline.

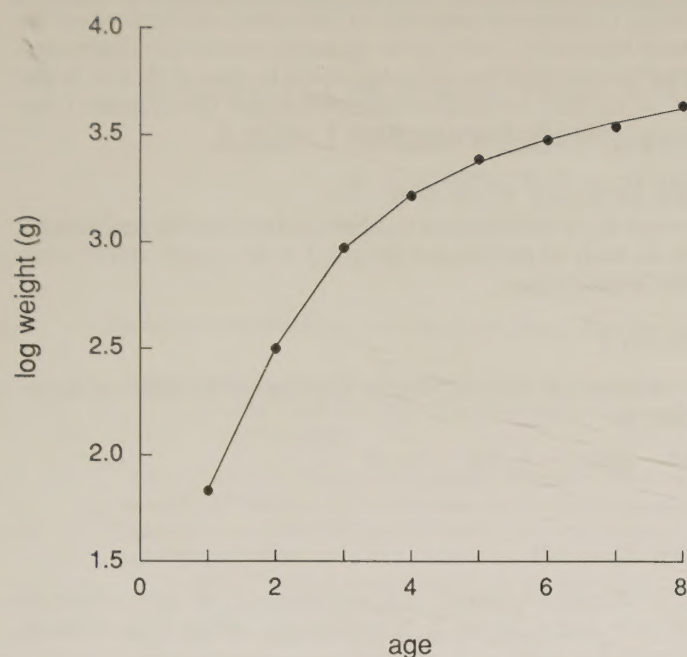


FIG. 1. Mean weight of Lake Ontario lake trout of various ages (solid symbols) and weight computed from the model using equation 5 (line).

TABLE 1. Average proportions (% by weight) of each prey species, and lake trout and prey lengths (l , cm) for different ages of lake trout

Age	Lake trout		Slimy sculpin		Rainbow smelt		Alewife	
	l	%	l	%	l	%	l	%
1	19.5	50	4.7	30	10.0	20	11.1	
2	32.0	40	5.7	40	11.0	20	12.2	
3	45.0	25	6.6	50	12.0	25	13.3	
4	54.2	10	7.3	40	12.7	50	14.2	
5	60.8	0	7.8	30	13.2	70	14.7	
6	65.7	0	8.1	30	13.6	70	15.2	
7	70.0	0	8.4	30	13.9	70	15.6	
8	73.1	0	8.7	30	14.2	70	15.9	

TABLE 2. Equations used to compute prey length from lake trout weight, and values of the coefficients used in these equations.

Equation	Source of data
$F_p = C_1 + 0.08 \cdot F_t$	Christie et al. 1987 (their fig. 9)
$\log(F_p) = \log(L_t) - 0.0356$	D. M. Whittle, unpubl. data
$\log(F_p) = \log(L_p) \cdot S + C_2$	D. M. Whittle, unpubl. data
$\log(W) = 3.14 \cdot \log(L_t) - 2.218$	D. M. Whittle, unpubl. data

where F_p = prey fork length (cm), F_t = lake trout fork length (cm), L_t = lake trout total length (cm), L_p = prey total length (cm), and W = lake trout weight (g)

Prey species	C_1	S	C_2
Slimy sculpin	3.3	1	0
Rainbow smelt	8.3	0.976	-0.0119
Alewife	8.3	0.943	0.0045

As lake trout grow, they appear to shift their diet from slimy sculpin to rainbow smelt and then to alewife (Christie et al. 1987). The average proportions of each prey species in the lake trout diet, as judged from the available literature and used in our model, are listed in Table 1.

The size of each prey species ingested, as a function of lake trout size, was estimated from fig 9 in Christie et al. (1987) using the relationships shown in Table 2. The lengths of slimy sculpin eaten by various sizes of lake trout, as computed from these equations, agree well with those reported by Elrod (1983), although the alewife are slightly longer. The lengths of alewife and rainbow smelt overlap those reported by Brandt (1986) for Lake Ontario and those computed using the equations given by Jude et al. (1987) for Lake Michigan. Jude et al. (1987) gave a steeper slope for the prey to predator length relationship for both prey species whereas Brandt (1986) did not find a positive prey to predator size correlation. The equations in Table 2, therefore, appear to provide reasonable average estimates of prey sizes.

Contaminant Concentration in Lake Trout and Their Prey

The observed contaminant concentrations in lake trout in 1986 are given by the regression

$$(6) \log(C) = \text{CONST} + \sum A_i D_i + b \cdot \log(W)$$

where CONST is a constant and A_i are the regression coefficients of each age, relative to age 4. D_i are dummy variables equal to 1 for age i and 0 for all others. The coefficients for equation 6 (Table 3) are the same as reported in Borgmann and Whittle (1991), except that CONST has been adjusted to give the mean concentration for sites 2, 7, 9, plus 11 in 1986, the only single year and sites for which data are available on all species.

Contaminant concentrations in rainbow smelt and slimy sculpin (Borgmann and Whittle 1992) were reanalyzed using the same regression model, but expressed as a function of body length rather than mass. This allows direct estimation of contaminant concentrations using prey length estimates (Table 2). Concentrations are given by

$$(7) \log(C) = \text{CONST} + b' \cdot \log(L_p)$$

Again, CONST was adjusted to give the mean concentration for sites 2, 7, 9, plus 11 in 1986.

Unfortunately, alewife contaminant concentrations were not monitored as a routine part of the contaminant surveillance program, but 43 alewife samples (five fish per sample) were analyzed in 1986 using the same methods as for rainbow smelt and slimy sculpin (Borgmann and Whittle 1992). The CONST and b' estimates for all three prey species are summarized in Table 3.

The contaminant concentration in each prey species increases with increasing age of lake trout because older lake trout consume larger prey and larger prey are more heavily contaminated (Table 3). Slimy sculpin, rainbow smelt, and alewife contain similar concentrations of mercury, and the average concentration in prey, therefore, increases with increasing lake trout age (Table 4). DDE and PCB concentrations in alewife, however, are lower than in slimy sculpin and rainbow smelt. Consequently, there is an increase in DDE and PCB concentration in prey from age 1 to 2 lake trout but a drop from age 3 to 5 as alewife become predominant in the lake trout diet. From age 5 on, average prey DDE and PCB levels again increase, as the mean prey size increases but species composition remains constant (Table 4). As a result of the interacting effects of prey species composition and contaminant concentrations in prey of different sizes, the average DDE and PCB concentrations in lake trout prey remain remarkably constant.

Lipid concentrations in lake trout and their prey were computed using the same procedures as for contaminant concentra-

TABLE 3. Values of constants (CONST), regression coefficients for lake trout age (A_i , relative to age 4), and the weight (b) or length (b') coefficients used in equations 6 and 7 for lake trout, slimy sculpin, rainbow smelt, and alewife.

Species	Variable	DDE	PCB	Hg	Lipid
Lake trout	CONST	-1.615	-0.669	-2.002	-0.398
	A_1	-0.381	-0.358	-0.134	-0.177
	A_2	-0.048	-0.108	-0.038	-0.042
	A_3	0.014	-0.001	-0.009	0.005
	A_4	0	0	0	0
	A_5	0.029	0.007	0.050	-0.017
	A_6	0.066	0.055	0.076	-0.015
	A_7	0.072	0.074	0.096	-0.038
	A_8	0.177	0.081	0.093	-0.013
	b	0.461	0.364	0.337	0.495
Slimy sculpin	CONST	-1.441	-0.665	-2.284	1.018
	b'	0.789	0.884	0.932	-0.119
Rainbow smelt	CONST	-2.678	-2.020	-3.418	0.169
	b'	1.775	1.628	1.718	0.462
Alewife	CONST	-5.120	-3.897	-5.215	3.344
	b'	3.353	2.868	3.217	-1.995

TABLE 4. Concentrations ($\mu\text{g/g}$ wet weight) of DDE, PCB, and mercury in lake trout of different ages and their prey in 1986 computed using the coefficients in Tables 1-3.

Lake trout age	DDE		PCB		Hg	
	Lake trout	Prey	Lake trout	Prey	Lake trout	Prey
1	0.0706	0.108	0.437	0.586	0.0303	0.0205
2	0.311	0.129	1.37	0.640	0.0638	0.0246
3	0.588	0.147	2.58	0.639	0.0979	0.0289
4	0.746	0.129	3.21	0.515	0.122	0.0320
5	0.941	0.112	3.71	0.410	0.154	0.0352
6	1.15	0.120	4.53	0.438	0.178	0.0382
7	1.27	0.127	5.07	0.461	0.198	0.0408
8	1.73	0.133	5.44	0.482	0.207	0.0430

TABLE 5. Lipid concentrations (%) and lipid-normalized concentrations ($\mu\text{g/g}$ lipid) of DDE and PCB in lake trout of different ages and their prey in 1986 computed using the coefficients in Tables 1-3.

Lake trout age	Lipid		DDE/lipid		PCB/lipid	
	Lake trout	Prey	Lake trout	Prey	Lake trout	Prey
1	2.15	9.31	3.29	1.69	20.3	8.12
2	6.33	8.25	4.92	2.14	21.6	9.46
3	12.0	7.62	4.91	2.57	21.6	10.1
4	15.8	8.37	4.71	2.18	20.2	8.02
5	18.2	8.70	5.18	1.79	20.4	6.15
6	20.6	8.31	5.56	1.92	22.0	6.59
7	21.5	8.01	5.91	2.03	23.6	6.98
8	24.5	7.77	7.06	2.13	22.2	7.33

tions (Table 5). Estimates of lipid concentrations in lake trout were used to test whether a better fit to the model could be obtained by setting contaminant elimination rates inversely proportional to lipid concentrations. Estimates of lipid concentrations in prey are not needed for Models 1 and 2, but were computed. Comparison of lipid-normalized DDE and PCB concentrations in lake trout and their prey demonstrates that there is food chain biomagnification of these contaminants, even after lipid normalization (Table 5). Lipid-normalized DDE concen-

trations increase only slowly with lake trout size and age whereas lipid-normalized PCB concentrations remain virtually constant throughout the age of the fish.

Contaminant Assimilation Efficiency (E_0)

E_0 was estimated to equal 0.67 for DDE (Niimi and Oliver 1988) and 0.75 for PCB (Niimi and Oliver 1983). The estimate for PCB is an average for a number of congeners. E_0 for mercury was assumed to equal 0.84, the assimilation efficiency of methylmercury (Boudou and Ribeyre 1985). Assimilation of methylmercury ranges from 70 to 90%, and the majority (usually greater than 80%) of mercury is in the methylated form in most freshwater fish tested (Huckabee et al. 1979). Absorption efficiencies of 0.8 for mercury and PCB are often assumed in contaminant models (Norstrom et al. 1976; Thomann and Connelly 1984).

Contaminant Elimination Rate (k_e)

There is insufficient data to allow estimation of k_e using our Model 1 (equation 2), but the coefficients a and n in Model 2 (equation 4) can be estimated. Norstrom et al. (1976) estimated n at 0.58 for methylmercury, and assumed that n for PCB was the same. Data compiled by Niimi (1987) suggest a decreasing k_e with increasing body size, but there is too much variability to estimate n accurately. We therefore assumed that n was equal to 0.58 for DDE also. Fitting equation 4 to the DDE data in Niimi (1987) with n set at 0.58 gives an estimate for the mean a of 0.033/d. The k_e for all the data, except one unusually short half-life of 4 d in a catfish, give an estimate for a of less than 0.075. This provides a maximum estimate for a . The PCB data summarized by Niimi (1987) are more variable than the DDE data, making estimation of a and n even more difficult. However, if n is again assumed to equal 0.58, then the data suggest that a is probably less than 0.1. The estimates of k_e for PCB and mercury are refined further as the model is fitted to the data.

Ingestion Rate

We assumed that ingestion was proportional to body size raised to some exponent. An allometric relationship between

body size and ingestion is often used in models, although it is usually applied to the maximum (ad libitum) ingestion rate and is temperature sensitive (e.g. Stewart et al. 1983; Barber et al. 1991). Preliminary estimates of the ingestion coefficients were made, and the changes in contaminant body burdens were computed (equations 1–4) over 1-d increments from age 1 to 8. The resulting predicted contaminant concentrations were compared with the observed concentrations, and the ingestion coefficients were adjusted accordingly. The process was repeated until the lowest sum of squares was obtained for the difference between the logarithms of the computed and observed concentrations.

Model Results

The computer model computations consisted of the following. First, daily iterations of growth were performed over the 7-yr period from age 1 to 8 using equation 5 to obtain body mass estimates at yearly intervals. Body lengths at these ages were computed (Table 2). The complete model was then run, again with daily iterations. Prey composition on each day was estimated from body length assuming a linear relationship between the fraction of each prey in the diet and body length (using prey composition estimates in Table 1) within any 1-yr period. Prey length was estimated using the equations in Table 2, and contaminant concentration in each prey species was computed using equation 7. Combined with prey composition estimates, this provided estimates of mean contaminant concentration in the diet. Total prey ingested was then estimated, from which total contaminant ingested was calculated. Lipid concentrations in lake trout were interpolated from concentrations at discrete ages using a log-log relationship between lipid and body mass. Contaminant elimination rates were then computed (equation 2 or 4). Finally, the increase in contaminant body burden was computed (equation 1 or 3) before proceeding to the next iteration.

DDE concentrations in lake trout were modelled first to provide estimates of ingestion rate because DDE was the most strongly biomagnified of the three contaminants. Daily ingestion rate as a fraction of body weight (I/W) was adjusted to give the best least squares fit to the log-transformed DDE concentrations. Three ingestion rate – body size relationships were tested: a constant I/W , I/W proportional to $W^{-0.2}$ which is roughly proportional to respiration rate, and I/W proportional to $W^{-0.1}$, an intermediate relationship. Three estimates of k_e were also used: a (equation 4) = 0, 0.033, and 0.075 representing minimum, mean, and maximum estimates of DDE clearance rates (with $n = 0.58$). All three ingestion rate – body size relationships gave good fits to the observed data, but the best was obtained using I/W proportional to $W^{-0.1}$. This relationship was used for all subsequent modelling. Ingestion rates (grams per gram per day) giving the best fit were 0.036, 0.044, and $0.055 \cdot W^{-0.1}$ for the minimum, mean, and maximum estimates of k_e , respectively. All three estimates of k_e resulted in good fits to the measured DDE concentrations, but the estimate of $a = 0.033$ was marginally the best ($R^2 = 0.997$). Prey ingestion rate by lake trout in 1986 was, therefore, probably about $0.044 \cdot W^{-0.1}/d$.

An estimate of the mean k_e for PCB was not obtained from the data in the literature, so this was computed using the ingestion rate estimated from the DDE data. The best estimate of k_e (again using $n = 0.58$) was obtained by setting $a = 0.060$. This is less than the maximum for a of 0.1 predicted from the literature. Alternatively, setting $a = 0.1$ (see above) gives an estimate of the maximum ingestion rate of $0.053 \cdot W^{-0.1}$, which

is close to the maximum ingestion predicted from the DDE data. The data on PCB and DDE concentrations in lake trout are, therefore, consistent with the slightly higher elimination rates for PCB suggested by the literature.

The above model, which assumes that k_e is related to body size (Model 2a), contains one of several possible equations for computing k_e . Lipid concentrations, however, increase rapidly with body size in lake trout, and it is equally possible that k_e is related to lipid concentrations, rather than to body size per se. Chlordane retention, for example, is directly proportional to lipid concentration (Roberts et al. 1977). Using the same ingestion rate ($0.044 \cdot W^{-0.1}$) and setting $k_e = \text{constant}/\% \text{lipid}$ (i.e. $n = 0$) gives just as good a fit to the observed data for both DDE and PCB (Model 2b, Table 6). These are two possible versions of Model 2 (equation 3). Fitting Model 1 (equations 1 and 2) to the data gives estimates of $K_{eq} \cdot f = 0.029$ for DDE and 0.047 for PCB. These are reasonable estimates ($R^2 = 0.991$ for DDE and 0.956 for PCB) but the sums of squares are three to four times larger than for the other models. However, setting $K_{eq} \cdot f$ inversely proportional to lipid concentrations results in a fit to the data which is as good as Model 2a or 2b (Model 1b, Table 6; Fig. 2). There are, therefore, at least three possible expressions for k_e which allow excellent prediction of DDE and PCB levels in lake trout, and it is not possible to distinguish between these based on the available data.

Fitting Model 2 to the mercury data, using the same ingestion rate and $n = 0.58$ as before, gave an estimate of $a = 0.21$, but the fit was rather poor ($R^2 = 0.849$). A much better fit was obtained by setting $n = 0$ and $a = 0.0027$ ($R^2 = 0.997$). A similarly good fit was obtained by setting $K_{eq} \cdot f = 0.16$ (Model 1a, Table 6; Fig. 2). There are, therefore, two expressions for k_e for mercury which give excellent predictions of concentrations in lake trout. Surprisingly, both these expressions predict that there is very little effect of body size on mercury elimination rate. The body size relationship ($n = 0.58$) used for DDE and PCB was obtained from studies on mercury elimination (Norstrom et al. 1976). However, some other studies have also shown no relationship between body size and mercury elimination, at least for inorganic mercury (Newman and Doubet 1989).

Implications of Ignoring Uptake from Water

The above analyses were conducted assuming that all contaminant uptake was from the food. If a significant amount of contaminant is taken up directly from the water, then the amount of food which must be ingested to produce a given body burden would be reduced, and our computed ingestion rates would be too high. An upper limit to the magnitude of this error can be computed. DDE and PCB concentrations on a lipid-normalized basis are higher in lake trout than in their prey (Table 5). This suggests a lack of chemical equilibrium between the DDE and PCB in the lipid of the fish and in the water and implies that excretion rates into the water should be greater than uptake rates from the water. The net chemical excretion rate (excretion minus uptake from water) should, therefore, be greater than 0, but somewhat less than the absolute chemical excretion rate. Consequently, the true prey ingestion rate should be less than $0.044 \cdot W^{-0.1}/d$ (obtained using k_e values listed in Table 6) but greater than $0.036 \cdot W^{-0.1}/d$ (obtained by setting k_e for DDE equal to 0). Ingestion could, therefore, be 0–18% lower than the ingestion rate used in our models, and direct uptake from water could account for between 0 and 18% of the total DDE uptake.

TABLE 6. Estimates of elimination rate coefficients giving the best fit to the contaminant concentrations measured in lake trout. Elimination rate (k_e) was computed as $E_0 \cdot K_{eq} \cdot f \cdot (I/W)$ in Model 1 and $a \cdot W^{-n}$ in Model 2. Ingestion rate (I/W , $\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$) = $0.044 \cdot W^{-0.1}$. E_0 = 0.67, 0.75, and 0.84 for DDE, PCB, and mercury, respectively. The range for k_e from age 1 to 8 is also shown.

Contaminant	Model	$K_{eq} \cdot f$	a (d^{-1})	n	k_e ($10^{-3}/\text{d}$)	R^2
DDE	2a	—	0.033	0.58	2.9–0.3	0.997
	2b	—	0.0067/%lipid	0	3.1–0.3	0.997
	1b	0.47/%lipid	—	—	4.2–0.2	0.997
PCB	2a	—	0.060	0.58	5.2–0.5	0.990
	2b	—	0.012/%lipid	0	5.6–0.5	0.989
	1b	0.79/%lipid	—	—	8.0–0.5	0.994
Hg	2a	—	0.0027	0	2.7	0.997
	1a	0.16	—	—	3.9–2.6	0.996

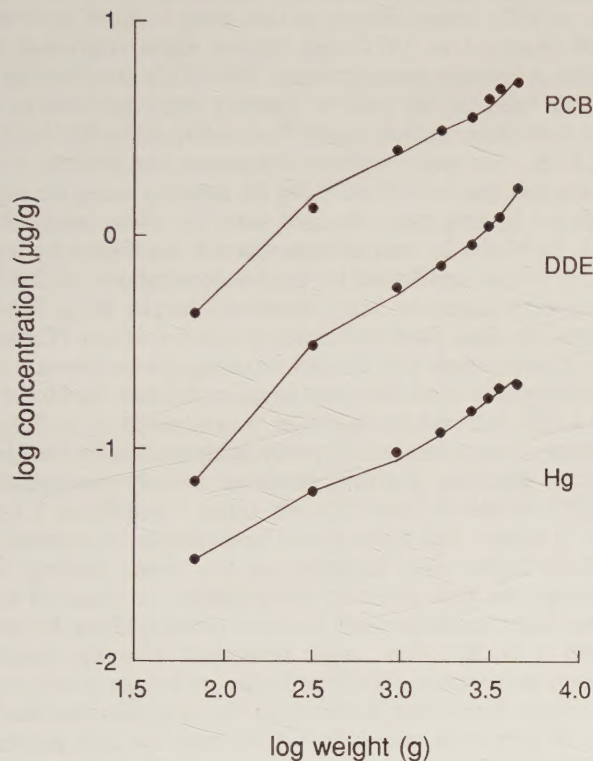


FIG. 2. Relationship between PCB, DDE, and mercury concentrations and body size for lake trout of different ages as obtained from the regressions in Borgmann and Whittle (1991) (solid symbols) and computed from Model 1.

Model Applications

Effect of Lipid Concentrations on DDE and PCB in Lake Trout

One of the most important applications of Models 1 and 2 is investigation of the relative importance of lipid concentrations in controlling DDE and PCB concentrations in lake trout. Circumstantial evidence suggests that lipid concentrations in lake trout affect biomagnification. Lipid levels appear to correlate with PCB concentrations across lakes in Ontario after correction for food chain length and latitude (Rasmussen et al. 1990). The ratio of DDE and PCB, but not mercury, concentrations in lake trout to those in rainbow smelt from Lake Ontario have been decreasing from 1977 to 1988 (Borgmann and Whittle 1992). This coincides with a decrease in lipid concentrations in lake trout (Borgmann and Whittle 1991). If DDE and PCB concentrations are normalized to lipid, then the

time trend in the lake trout to rainbow smelt ratio disappears (Borgmann and Whittle 1992). Furthermore, Models 1 and 2 provide a good fit to the observed concentrations if elimination rates of DDE and PCB, but not of mercury, are related to lipid concentrations. This suggests that part of the decrease in DDE and PCB concentrations in lake trout may have been caused by the lower lipid levels, and hence greater excretion rates. To test this possibility, the models were run with all parameters set as before, except for CONST for lipid, which was set equal to either -0.274 or -0.443 , giving lipid concentrations observed in 1977 or 1988, respectively.

The responses of Models 2a, 2b, and 1b (Table 6) to a change in lipid concentrations are different. Biomagnification is not affected by lipid concentrations in Model 2a because k_e is a function of body size alone. Use of Model 2b or 1b resulted in DDE concentrations from 6 to 9% higher and PCB concentrations from 10 to 15% higher, depending on age, in 1977 than in 1988. By comparison, lipid concentrations were 48% higher in 1977 than 1988. For example, DDE concentrations in age 4 lake trout were predicted to be 0.80 (1977), 0.77 (1986), and 0.75 $\mu\text{g/g}$ (1988) at lipid concentrations of 21.0, 15.8, and 14.2% using Model 1b, even though DDE excretion is inversely proportional to lipid concentrations in the model. The response of DDE or PCB concentrations in lake trout to changing lipid concentrations is relatively small because excretion rates, although inversely proportional to lipid in Models 2b and 1b, are slow and have only a limited impact on final concentrations. Much of the control over final concentration is the result of growth dilution. For example, DDE elimination rates (Table 6) are lower than growth rates (4.5×10^{-3} to $0.35 \times 10^{-3}/\text{d}$, equation 5) for all ages of lake trout. For age 4 fish, k_e for DDE equals $0.4 \times 10^{-3}/\text{d}$ compared with a growth rate of $1.2 \times 10^{-3}/\text{d}$. Growth dilution, therefore, plays a greater role than lipid concentration in controlling final DDE concentrations. It is not, therefore, appropriate to normalize DDE or PCB levels to lipid when investigating biomagnification of these compounds in lake trout.

The decreasing growth rate with increasing age, combined with the low excretion rates of DDE and PCB, results in a rapid increase in contaminant concentration with age in lake trout (Table 4). Lipid concentrations also increase rapidly with age, resulting in a much smaller change in lipid-normalized DDE or PCB concentrations (Table 5). In view of the above analysis, however, the uniformity in lipid-normalized concentrations with age would appear to be coincidental, and not a result of the increasing lipid concentrations. In the three prey species,

TABLE 7. Relative changes in ingestion rate required to increase PCB concentrations from 1986 to 1977 levels (a 2.2-fold increase), and accompanying changes in excretion rates of PCB and mercury and steady-state mercury concentrations using Models 2b and 1b for PCB and Models 2a and 1a for mercury.

Parameter	Relative change	
	Model 2	Model 1
I	2.0	3.4
k_e (PCB)	0.75	2.6
k_e (Hg)	1.0	3.4
[Hg]	2.0	1.2–1.7

in contrast, DDE and PCB concentrations on a lipid-normalized basis increase with increasing body size because lipid concentrations either decrease, or increase more slowly than DDE and PCB with increasing body length (Table 3).

Effect of Varying Ingestion Rates

The above analyses raise an important question. PCB levels in both rainbow smelt and slimy sculpin have not declined appreciably from 1977 to 1988 (Borgmann and Whittle 1992) whereas concentrations in lake trout appear to have gone down (Borgmann and Whittle 1991). Changes in lipid concentrations in lake trout do not fully explain this discrepancy. What, then, is the cause of the decrease in PCB concentrations in lake trout? There are at least two possibilities. First, PCB concentrations in alewife might have decreased substantially or the diet of lake trout might have shifted from predominantly rainbow smelt in the early years to the less contaminated alewife in later years. A decrease in PCB concentrations in alewife is possible, but seems surprising if concentrations in rainbow smelt and slimy sculpin have not changed. We have no data between 1977 and 1988 indicating a change in diet, although this possibility cannot be excluded.

A second possibility is that the amount of prey ingested by the lake trout has dropped. A higher ingestion rate in the earlier years might be the reason for both the higher lipid and PCB concentrations. Growth rate does not appear to have been affected significantly because the average size of various ages of fish has not decreased noticeably (Borgmann and Whittle 1991). The increased ingestion could have resulted in increased lipid storage and would have resulted in a decreased growth efficiency and hence higher PCB concentration. Similarly, Hamelink and Spacie (1977) argued that correlations between contaminant concentrations and lipid content did not necessarily prove a cause and effect relationship, but that factors (especially feeding) which result in lipid storage may also promote contaminant accumulation. The same phenomenon might also explain the apparent correlation between lipid and PCB concentrations in lake trout across lakes in Ontario, even though concentrations are mainly controlled by food chain biomagnification rather than lipid–water partitioning (Rasmussen et al. 1990). To test this possibility, Models 1b and 2b were run using the 1977 lipid concentrations, but with increasing ingestion rates until PCB concentrations matched those observed in 1977 (a 2.2-fold increase over 1986). Using Model 2b, the predicted ingestion rate in 1977 would have been $0.089 \cdot W^{-0.1}$. The predicted increase in ingestion (a 2.0-fold increase) is slightly less than the increase in PCB concentration because the higher lipid levels result in a 25% lower PCB excretion rate (Table 7). Alternatively, using Model 1b, the predicted ingestion rate in 1977 would have been 3.4-fold higher than in 1986. In this

model, excretion is linked to ingestion. The k_e is therefore higher, although only 2.6-fold, less so than the 3.4-fold increase in ingestion rate, because of the higher lipid concentrations. Models 2b and 1b, therefore, provide different estimates of the increase in feeding rate required to raise PCB concentrations to those observed in 1977.

The above calculations are based on the assumption that assimilation efficiency remains constant and is not a function of ingestion rate. If biomass assimilation efficiency decreases with increasing ingestion rate, then $f(= (I - A)/I)$ increases and k_e could increase even further (equation 2). This would make the differences between Models 1 and 2 even greater than suggested in Table 7. Assimilation efficiency was held constant in our model because we did not have any data on how this varies with ingestion rates for lake trout in Lake Ontario.

Although a higher feeding rate might help explain the higher ratio of PCB concentrations in lake trout to those in rainbow smelt observed in 1977, this creates some confusion with respect to mercury concentrations. Should a higher feeding rate not also increase the ratio of mercury concentrations in lake trout to those in rainbow smelt? This ratio, unlike that for DDE and PCB, was quite constant (Borgmann and Whittle 1992). To test this, the models were run for mercury using the higher predicted feeding rates obtained from the above analysis for PCB. In Model 2a, mercury excretion is unaffected by ingestion. It is also unaffected by lipid concentrations. A 2.0-fold increase in ingestion rate, therefore, results in a 2.0-fold increase in final predicted mercury concentrations (Table 7). This is inconsistent with the lack of change in the mercury concentration ratio from lake trout to rainbow smelt. In Model 1a, a 3.4-fold increase in ingestion would result in a 3.4-fold increase in excretion, which partly compensates for the higher mercury ingestion. The final predicted mercury concentrations in 1977 would be from 1.2-fold (ages 7 and 8) to 1.7-fold (age 2) higher. Age 4 fish would have mercury concentrations 1.4-fold higher than expected at the lower feeding rate. Although the final mercury concentration is expected to be higher, the increase is much less than predicted from Model 2. Model 1 is, therefore, more consistent with the observed changes in the ratios of PCB and mercury in lake trout to those in rainbow smelt than is Model 2. This suggests that feeding rates of lake trout may indeed have been 3.4-fold greater in 1977 than in 1986.

Comparison of predicted and observed concentrations of PCB and mercury in lake trout and rainbow smelt, therefore, suggests that Model 1, where contaminant elimination is closely associated with defecation, is superior to Model 2. However, this is based on the assumption that PCB and mercury concentrations in alewife remained roughly proportional to those in rainbow smelt from 1977 to 1986. Since data on PCB and mercury concentrations in alewife prior to 1986 are not available, Model 2 may still prove to be valid.

Effect of Diet Composition on Contaminant Concentrations

An important question is: what would be the change in contaminant concentrations in lake trout if the prey diet changed due to the collapse of one or two of the prey species populations? There has recently been a major collapse in the alewife population of Lake Michigan (Jude and Tesar 1985), and a similar shift in prey populations in Lake Ontario is possible. The model predictions of lake trout contaminant concentrations for lake trout feeding entirely on a single prey species, using Model 1 (Model 2 predictions are virtually identical), are shown in Fig. 3. Exclusive feeding on rainbow smelt or slimy sculpin

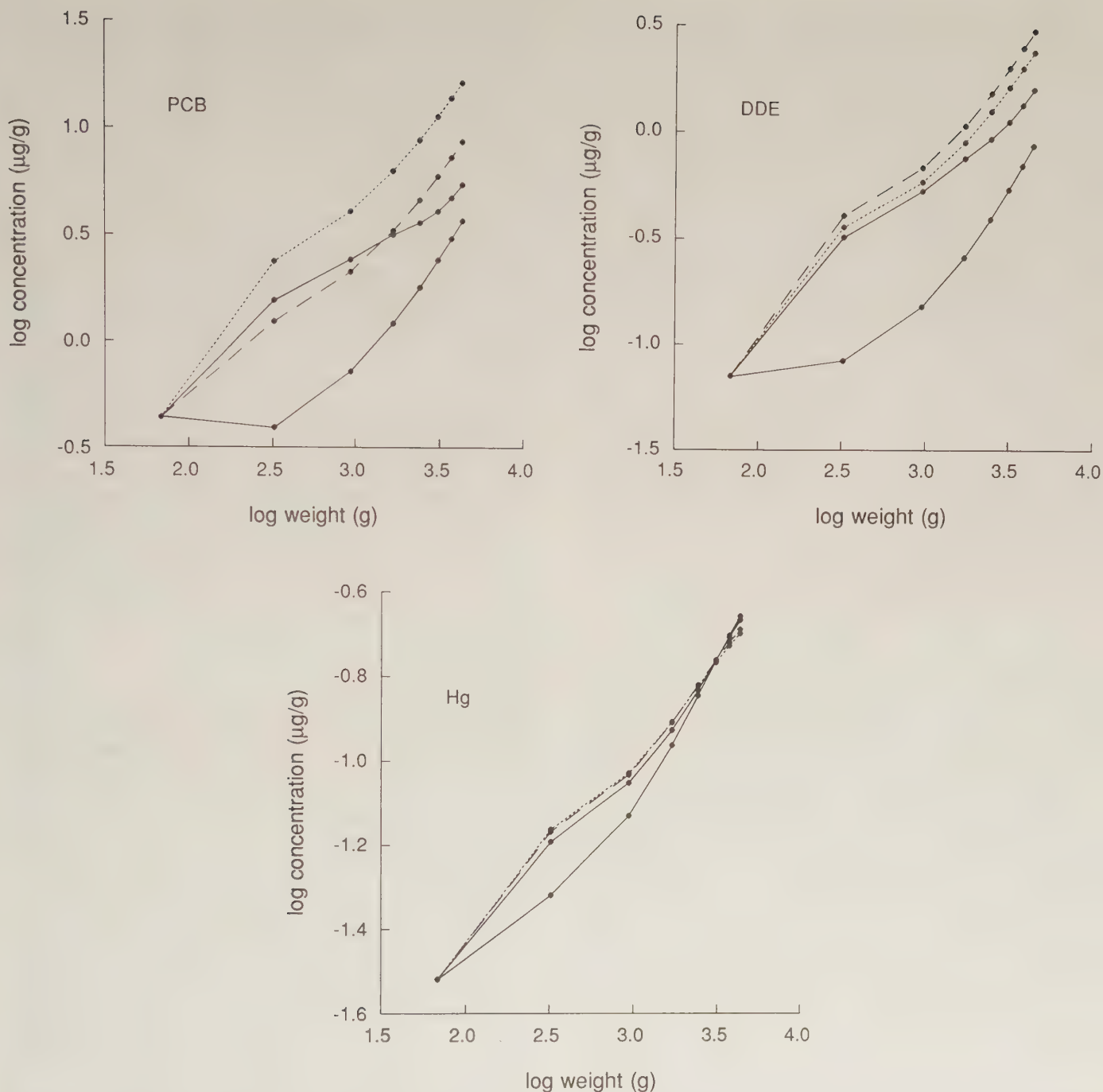


FIG. 3. Concentrations of PCB, DDE, and mercury in lake trout computed from Model 1 assuming prey consisted of the three forage fish species in the proportions listed in Table 1 (upper solid line), alewife only (lower solid line), rainbow smelt only (broken line), or slimy sculpin only (dotted line).

would result in higher DDE concentrations whereas feeding on alewife alone would substantially reduce DDE concentrations. PCB concentrations would also be reduced if lake trout fed exclusively on alewife and increased if they fed entirely on slimy sculpin. If they fed mostly on rainbow smelt, however PCB concentrations would be slightly lower in 2- and 3-yr-old lake trout and slightly higher in 5- to 8-yr-old fish. Figure 3 shows that the shift in prey from slimy sculpin to rainbow smelt to alewife is responsible for a reduction in the slope of the PCB – body size relationship, relative to what would occur if prey consisted of a single species. Perhaps a change in prey species selection towards the less contaminated alewife also

contributed to the decline in PCB and DDE concentrations in lake trout from 1977 to 1988.

A change in prey species composition would have little effect on mercury concentrations in lake trout because mercury concentrations in the three prey species are relatively uniform. The only difference would be a reduced concentration in 2- and 3-yr-old fish feeding exclusively on alewife (Fig. 3).

Rate of Response to Changing Concentrations in the Diet

Another important question is: how fast do concentrations in lake trout respond to changes in concentrations in the prey? Deviations in PCB concentrations in lake trout about the gen-

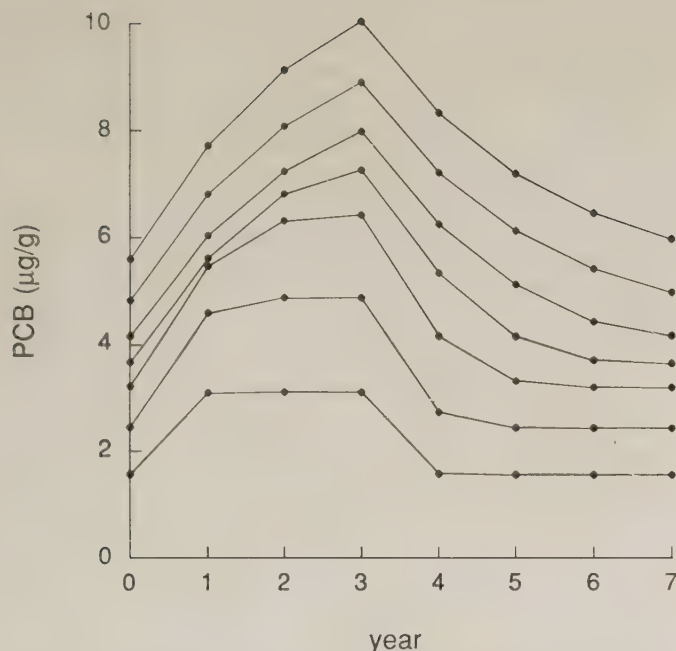


FIG. 4. Changes in PCB concentrations in age 2–8 lake trout with time following a sudden doubling of PCB concentrations in the prey at year 0, constant prey PCB concentrations for 3 yr, and a sudden return in prey PCB concentrations to initial levels in year 3.

eral time trend correlate closely with the rate of increase in alewife populations (Borgmann and Whittle 1991). This suggests that concentrations in lake trout respond rapidly to changes in prey population dynamics, presumably as a result of changes in prey contaminant concentrations. Is this possible in a long-lived fish such as the lake trout?

To demonstrate the effect of a sudden change in prey concentration, such as might have occurred for PCB (Borgmann and Whittle 1991), predicted concentrations of PCB in lake trout of different ages following a sudden doubling of PCB concentrations in the prey for 3 yr, followed by a sudden drop back to the original concentration, were modelled (Fig. 4). Age 2 and 3 fish followed the change in prey concentration almost immediately. There was a slight delay in older fish. However, the peak in even the age 8 fish was delayed by only about 1 yr. The lake trout can, therefore, respond rapidly to changes in concentrations in their prey. This is partly due to contaminant elimination (k_e), but also largely due to growth dilution and the aging of the fish (e.g. age 4 fish were age 3 fish the previous year and age 3 fish respond faster than age 4 fish). This explains why the year to year changes in PCB concentrations for all ages of lake trout are roughly similar and allows the use of a regression model which does not require an interaction term between fish age, contaminant concentration, and year (Borgmann and Whittle 1991).

Comparison with Other Models

Our models and calculations differ from most in the literature in several respects. First, they utilize an unusually large data set (over 1800 PCB and DDE and over 1600 mercury determinations in lake trout; Borgmann and Whittle 1991). The result is a fairly smooth curve in the observed age-specific contaminant concentration – body size relationship to which the model is fitted (Fig. 2). Second, Model 1 used the assumption that contaminants diffuse across the intestinal wall down concen-

tration gradients, and this controls assimilation efficiency and depuration. Thermodynamic equilibrium across the intestine is considered in relatively few models (Gobas et al. 1988; Barber et al. 1991). Usually, contaminant elimination is assumed to be an exponential function of body size (Norstrom et al. 1976; Jensen et al. 1982), as in our Model 2. Although practical, this approach is much less satisfying thermodynamically (Barber et al. 1991).

The major difference between our modelling approach and most others, however, is that we estimated ingestion directly from contaminant concentrations in predator and prey. Niimi (1981) and Devaux and Monod (1987) published earlier attempts at using PCB and DDE concentrations as bioenergetic indicators in lake trout and brown trout (*Salmo trutta*), respectively. In most models, however, whether applied to contaminant bioaccumulation or simply to bioenergetics, ingestion is estimated by first estimating metabolism and then calculating the sum of metabolism and growth and correcting for assimilation efficiency and other minor losses (Norstrom et al. 1976; Jensen et al. 1982; Stewart et al. 1983; Thomann and Connolly 1984; Barber et al. 1991).

Estimates of metabolism are not needed in our modelling approach, since ingestion and growth are determined independently, but metabolism and biomass conversion efficiency can be calculated from the model's output. This is a useful exercise because it provides an estimate of metabolism under field conditions, and considerable effort often goes into calculating this parameter in models (e.g. Norstrom et al. 1976; Stewart et al. 1983). If we assume a biomass assimilation efficiency of 80%, then food used to meet metabolic requirements drops from 0.018 to 0.015/d from age 1 to age 8 (Table 8). This is a little higher than metabolic requirements for Lake Michigan lake trout estimated by Thomann and Connolly (1984, their fig. 1: 0.01/d for a 100-g fish) and varies less with body weight. A higher metabolic rate would result in a lower biomass conversion efficiency. Our biomass conversion efficiencies are lower than those predicted for lake trout in Lake Michigan by Stewart et al. (1983: 0.24 at age 1 to 0.074 by age 9). Our model predicts that most of the assimilated biomass goes into metabolism, and not growth, by age 8. These higher ingestion and metabolic rates are required in order to raise DDE and PCB concentrations in older lake trout to the observed levels, based on contaminant concentrations in alewife. As indicated above, if direct uptake of DDE and PCB from water contributes significantly to the body burden, then our estimates of ingestion rates will be too high and conversion efficiencies too low. Correction for the unaccounted uptake from water, however, should reduce the estimated ingestion rates by less than 18%.

Conclusions

Contaminant concentrations in lake trout and their prey can be used to estimate ingestion rates and produce a bioenergetics model. This model can then, in turn, be used to examine how different factors control the degree of contaminant biomagnification. DDE is the most useful bioenergetics marker in such a model because it has the lowest excretion rates of the three contaminants studied. A large proportional error in the estimate of DDE clearance will, therefore, have a relatively minor effect on predicted ingestion rates. Mercury is a less useful marker because of its higher excretion rate and the lack of published data on the relationship between mercury excretion and body size in lake trout.

Concentrations of PCB, DDE, and mercury in lake trout respond rapidly to changes in the concentration of their food.

TABLE 8. Weight and instantaneous and total annual integrated growth, ingestion, and biomass conversion efficiency (K_1) predicted from an ingestion rate of $0.044 \cdot W^{-0.1}$ and the growth curve used in the model. Also shown is the food biomass used to meet metabolic requirements assuming a biomass assimilation efficiency of 80%.

Age	Weight (g)	Instantaneous			Metabolism (d ⁻¹)	Integrated		
		Growth (d ⁻¹)	Ingestion (d ⁻¹)	K_1 (%)		Growth (g/yr)	Ingestion (g/yr)	K_1 (%)
1	68	0.00455	0.0289	15.8	0.0185	254	1 592	15.9
2	322	0.00377	0.0247	15.2	0.0160	617	5 099	12.1
3	939	0.00214	0.0222	9.6	0.0156	751	10 266	7.3
4	1690	0.00121	0.0209	5.8	0.0155	724	15 382	4.7
5	2414	0.00079	0.0202	3.9	0.0154	676	20 033	3.4
6	3090	0.00058	0.0197	2.9	0.0152	633	24 268	2.6
7	3723	0.00045	0.0193	2.3	0.0150	597	28 172	2.1
8	4320	0.00037	0.0191	1.9	0.0149			

This is due largely to fish growth, rather than contaminant excretion, especially for PCB and DDE. Consequently, lipid concentrations in lake trout have only a modest effect on final PCB and DDE concentrations, even if elimination rates are inversely proportional to lipid concentration. It is not, therefore, appropriate to normalize PCB and DDE concentrations to lipid when examining contaminant trends in lake trout.

PCB concentrations in lake trout have decreased from 1977 to 1988, but those in rainbow smelt and slimy sculpin have not. A possible explanation is that prey ingestion rates have decreased, but not sufficiently to reduce growth rates, resulting in increased biomass conversion efficiencies and reduced food chain biomagnification. The decrease in lipid concentrations supports this conclusion. Another alternative would be a gradual decrease in PCB concentrations in alewife relative to that in rainbow smelt and slimy sculpin. This decrease would have to be superimposed on the short-term contaminant fluctuations in alewife likely to occur as a result of oscillations in alewife population biomass and hypothesized to be responsible for the oscillations in PCB concentrations in lake trout (Borgmann and Whittle 1991). In order to better understand these interactions, alewife should be included in future contaminant monitoring programs. The prey species in the gut of lake trout should also be monitored, so that the effect of changes in diet composition on contaminants ingested can be evaluated.

Final contaminant concentrations in lake trout are a complex interplay of both bioenergetic changes in the lake trout and changes in the population structure of their prey. Without an understanding of these interactive effects, it is dangerous to use concentrations of contaminants in the lake trout as indicators of the degree of contamination of the ecosystem as a whole and the efficacy of various remedial actions. For example, PCB concentrations in lake trout have been declining with a half-life of about 10 yr (Borgmann and Whittle 1991), suggesting that Lake Ontario is gradually becoming cleaner. However, PCB concentrations in rainbow smelt and slimy sculpin have not declined appreciably (Borgmann and Whittle 1992), and the above models suggest that this discrepancy may be due to a reduction in lake trout ingestion rates. This raises an important question: has the degree of PCB contamination in Lake Ontario really gone down, or is the apparent improvement in lake trout PCBs concentrations due primarily to changes in the food web and the degree of PCB biomagnification? This question must be addressed in future monitoring programs.

Acknowledgments

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Appendix

The expression for contaminant elimination rate in the absence of uptake from, or excretion directly into the water (equation 2 in the text) is derived below. The rate of change in contaminant burden in the gut (B_g) is given by

$$(1A) \quad dB_g/dt = I \cdot C_i - F \cdot C_g + k_b \cdot C_b \cdot W - k_g \cdot C_g \cdot W$$

where k_b and k_g are the rate constants (per day) for contaminant passing from the body into the gut (gastrointestinal tract) and from the gut into the body, expressed on a per unit body mass basis. C_i , C_g , and C_b are the concentrations of the contaminant in the ingested food, gut, and body, respectively, and W is body mass. The rate of change in the volume of the gut contents is given by

$$(2A) \quad dG/dt = I - F - A$$

where A is assimilation. Differentiation of $C_g = B_g/G$ at steady state ($dC_g/dt = 0$) gives

$$(3A) \quad C_g \cdot dG/dt = dB_g/dt.$$

Substitution of equations 1A and 2A and simplification gives

$$(4A) \quad C_g = \frac{I \cdot C_i + k_b \cdot C_b \cdot W}{I - A + k_g \cdot W}.$$

The net assimilation efficiency of the contaminant is given by

$$(5A) \quad E = \frac{I \cdot C_i - F \cdot C_g - dB_g/dt}{I \cdot C_i} = \frac{k_g \cdot C_g \cdot W - k_b \cdot C_b \cdot W}{I \cdot C_i}.$$

Substituting equation 4A into equation 5A and simplifying gives

$$(6A) \quad E = \frac{k_g \cdot W}{I - A + k_g \cdot W} \left[1 + \frac{k_b \cdot C_b \cdot W}{I \cdot C_i} \right] - \frac{k_b \cdot C_b \cdot W}{I \cdot C_i}.$$

If the contaminant concentration in the body of the fish is approximately 0, such as at the beginning of an experiment on contaminant uptake from food, then the initial assimilation efficiency (E_0) is

$$(7A) \quad E_0 = \frac{k_g \cdot W}{I - A + k_g \cdot W}$$

which can also be written as

$$(8A) \quad k_g \cdot W = \frac{E_0 \cdot (I - A)}{1 - E_0}.$$

If $I - A$ is set equal to F ($dG/dt = 0$), then equations 4A, 5A, and 7A are equivalent to equations 17b, 21, and 22 of Gobas et al. (1988) but written in terms of concentrations and rate constants rather than fugacity. Substituting equation 7A into equation 6A and simplifying gives

$$(9A) \quad E = E_0 - (1 - E_0) \frac{k_b \cdot C_b \cdot W}{I \cdot C_i}.$$

The equilibrium constant for contaminant flow between the gastrointestinal tract and the fish is

$$(10A) \quad K_{eq} = k_b/k_g \text{ or } k_b = K_{eq} \cdot k_g.$$

Substituting equation 10A into equation 9A and simplifying using equation 8A gives

$$(11A) \quad E = E_0 [1 - K_{eq} \cdot f \cdot (C_b/C_i)]$$

where $f = (I - A)/I$. The rate of change in the total body burden (B) of contaminant, assuming no direct excretion into the water through the gills or urine and no metabolic breakdown of contaminant, is

$$(12A) \quad dB/dt = E \cdot I \cdot C_i = E_0 \cdot I \cdot C_i - E_0 \cdot K_{eq} \cdot f \cdot C_b \cdot I.$$

Using $C_b = B/W$ and simplifying gives

$$(13A) \quad \begin{aligned} dB/dt &= E_0 \cdot I \cdot C_i - E_0 \cdot K_{eq} \cdot f \cdot (I/W) \cdot B \\ &= E_0 \cdot I \cdot C_i - k_e \cdot B \end{aligned}$$

where

$$(14A) \quad k_e = E_0 \cdot K_{eq} \cdot f \cdot (I/W).$$

This is equation 2 in the text.

Seawater Adaptability in Baltic Salmon (*Salmo salar*): A Bimodal Smoltification Pattern in Previously Mature Males

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In three years, maximum seawater adaptability in 2-yr-old Baltic salmon (*Salmo salar*) was found during a period of about 2 wk in late May – early June. At this time the previously mature males developed in one of two directions. One group (8–44%) developed a seawater adaptability comparable with that of immature fish whereas the other did not. Previously mature males that did not adapt to seawater had larger gonadosomatic indices than males with good performance in seawater. Individually tagged previously mature males and immature fish released into the river in spring had a total adult recapture rate of 1.6 and 11.6%, respectively. In both groups the recapture rate was highest for fish released about 2 wk before the time of optimal seawater adaptability. The effect of size on recapture rate was more pronounced among previously mature males than among immature fish, with a particularly low recapture rate of small previously mature males. It is suggested that previously mature male parr can either mature sexually again or complete smoltification in spring and that the probability for smoltification and seaward migration is affected by body size and the conditions for growth during the previous autumn–winter.

En trois années, on a observé que l'adaptabilité maximale à l'eau salée chez le saumon de l'Atlantique (*Salmo salar*) âgé de 2 ans s'étale sur une période d'environ 2 sem, à la fin de mai ou au début de juin. À cette époque, on a enregistré deux types de comportement chez les mâles déjà rendus à maturité. Le premier groupe (entre 8 et 44 %), contrairement au deuxième, a démontré une adaptabilité à l'eau salée comparable à celle du poisson immature. Les mâles déjà rendus à maturité qui ne se sont pas adaptés à l'eau salée avaient des indices gonadosomatiques plus élevés que les mâles qui se sont bien acclimatés à l'eau salée. Des mâles déjà rendus à maturité et des saumons immatures ont été étiquetés individuellement et relâchés dans la rivière au printemps; les taux de recapture de spécimens adultes se situaient à 1,6 et à 11,6 % respectivement. Dans les deux cas, le taux de recapture était plus élevé pour les poissons relâchés environ 2 sem avant la période optimale d'adaptabilité à l'eau salée. L'incidence de la taille sur le taux de recapture était plus marquée chez les saumons déjà rendus à maturité que chez les autres, le taux de recapture étant particulièrement faible chez les spécimens de petite taille appartenant au premier groupe. Selon nous, les tacons mâles qui sont déjà rendus à maturité peuvent, soit atteindre de nouveau leur maturité sexuelle, soit terminer leur smoltification au printemps, la probabilité de smoltification et de migration vers la mer étant tributaire de la taille du poisson et des conditions de croissance prévalant au cours de l'automne et de l'hiver précédant l'arrivée en eau salée.

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Sexual maturation is commonly observed among wild and hatchery-propagated male Atlantic salmon (*Salmo salar*) parr (Mitans 1973; Thorpe and Morgan 1980; Saunders et al. 1982; Dalley et al. 1983; Lundqvist 1983). Even if mature parr may smoltify successfully the following spring (Mitans 1973; Thorpe and Morgan 1980; Saunders et al. 1982), there is evidence from natural stocks that they do not migrate to sea in the same number as sexually immature fish of the same age (Österdahl 1969; Dalley et al. 1983; Myers 1984; Berglund et al. 1991a). Also, previously mature male parr from hatchery stocks released into the river during the smolt migration are less likely to migrate to sea (Hansen et al. 1989), and they may contribute significantly less to the sea-catch of salmon than do previously immature fish (Eriksson et al. 1987; Lundqvist et al. 1988). However, Hansen (1980) found no differences in recapture rate between releases of smolts from the two categories.

Some studies have reported that previously mature male parr have a poor hypoosmoregulatory capacity (Lundqvist et al. 1989) and low gill Na^+, K^+ -ATPase activity (Langdon and Thorpe 1985; Birt and Green 1986) compared with nonmaturing fish during the smoltification period. Other studies have

reported that gill Na^+, K^+ -ATPase activity and survival in full-strength seawater are as high in previously mature males as in immature fish (Saunders et al. 1982).

Thus, at present there are contradictory conclusions concerning the effect of early sexual maturation on smoltification in male salmon parr. This study was designed to examine the effect of sexual maturation, body size, and time on seawater adaptability in a Baltic stock of Atlantic salmon. This was done by periodically challenging groups of previously mature male parr and immature fish in seawater during spring in three different years to assess their seawater adaptability. In one year, corresponding groups of fish were released in the river and the adult recapture rates were analysed.

Material and Methods

Fish

Two-year-old Atlantic salmon parr, of Baltic origin, belonging to the Umeälven (Ume River) stock were used in this study. The fish were raised at the Norrfors Hatchery (63°N, 20°E) under standard hatchery conditions, at natural

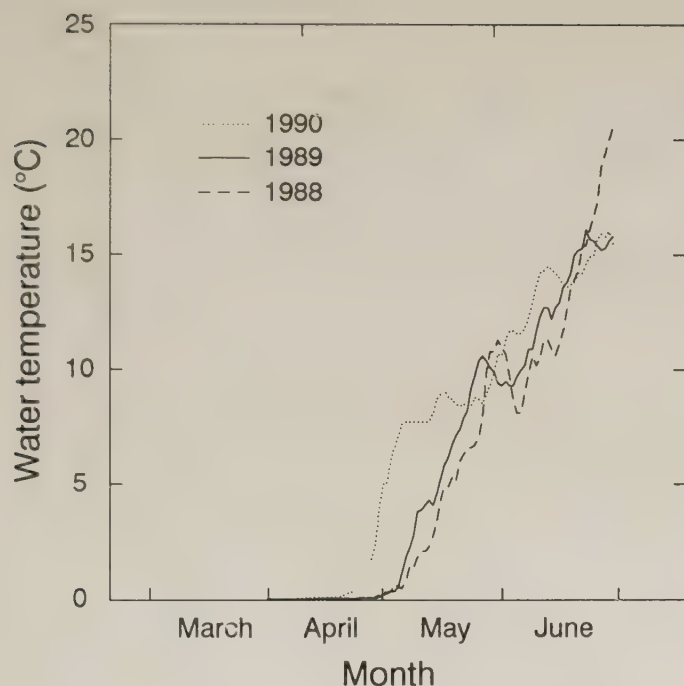


FIG. 1. Ambient water temperature during spring 1988–90.

photoperiod, with through-flowing river water at ambient temperature (Fig. 1). In midwinter, salmon parr in the size range (12–20 cm) of potential smolts were sampled randomly from large 95-m² stock tanks. By that time, two groups were identifiable: mature males with running milt and immature fish of both sexes.

Seawater Adaptability

At five times (5 May, 15 May, 27 May, 9 June, and 21 June) in spring, 36 individuals from each group were challenged in 25‰ artificial seawater (HW-Marinemix) for 24 h to assess their seawater adaptability (Blackburn and Clarke 1987). These dates were chosen because they cover the period of the natural smolt migration in this part of Sweden (Österdahl 1969). The experimental design was repeated for three successive years (1988, 1989, and 1990). The fish were tested at ambient river water temperature (Fig. 1) and were not fed during the tests. During the tests the artificial seawater was filtered, aerated, and circulated by a pump. After 24 h the fish were anaesthetized in 2-phenoxyethanol (0.5 mL/L), weighed (0.1 g), measured (0.1 cm), and blood samples taken by severing the caudal peduncle. The blood was collected in heparinized hematocrit tubes, centrifuged, and the plasma collected and stored at –20°C. The plasma sodium levels were measured by atomic absorption spectrophotometry. After the blood was taken the fish were dissected and sexed. In males the gonads were weighed (0.01 g) and notes were taken as to whether the gonads were regressing or had changed external appearance and texture and formed a firm swelling tissue typical of gonads in an early stage of maturation.

Recapture Rates

In spring 1988, 10 000 previously mature males and 5000 immature fish, distributed within the size range of 13–19 cm (nearest 0.5 cm), were individually identified with external tags (Carlin 1959). Groups of fish, 2000 previously mature males and 1000 immature fish, were released in the River Umeälven

on the same five dates that the seawater challenge tests (above) were done. On each date, about 15 000 nontagged fish were released together with the tagged fish.

Adult recaptures were obtained from the open-sea fishery in the Baltic sea or as spawning migrants along the coast in the Gulf of Bothnia or in their home river. Tag return data were entered into computer files at the Salmon Research Institute, Älvkarleby, Sweden. The recapture data reported in this paper are from smolt releases in 1988 and include tag return data from June 1988 to August 1991. As very few fish survive their third sea-winter in the Baltic, the recapture rates reported in this paper are close to 100% of the true figures. This recapture experiment is part of a larger study that will be completed in 1993.

Data Analysis

To study the distributional patterns of data, we used the probability plotting (*p*-plot) technique (Sokal and Rohlf 1981; Chambers et al. 1983) when analysing the plasma sodium values after seawater challenge. When the expected normal quantiles are plotted against the ordered observations, samples from a normal distribution will lie on a straight line. A bimodal distribution will form a curved line with an inflection point (cf. Cassie 1954). Epanechnikov kernel density estimators (Silverman 1986; Wilkinson 1990) and Spearman's rank correlation were used to examine the relationship between plasma sodium values and gonadosomatic index ($GSI = 100(\text{gonad weight} / \text{total body weight})$). The seasonal changes in seawater adaptability in each year were analysed with ANOVA. For this purpose, we used data from immature females only, thus avoiding the effects of sexual maturation on seawater adaptability in males.

Probit models were used to examine the relationship between recapture rate, data of release, and fish fork length at tagging. As previously mature males >18 cm were rare, only fish <18 cm were included in the analysis. Early inspection of the recapture data by distance-weighted least squares smoothing (McKlein 1972; Wilkinson 1990) suggested that the recapture rate was positively related to fish length and showed a quadratic relation to date of release. Therefore, a probit model of the following form was fitted to the data:

$$\text{Prob}_{(\text{recap})} = \alpha + \beta_1 d + \beta_2 x_1 + \beta_3 x_2 + \beta_4 y + \beta_5 y^2$$

where α is a constant, d is a dummy variable taking the value 1 for immature fish and 0 for previously mature males, x_1 is d times fish length at tagging, x_2 is $1 - d$ times fish length at tagging, y is release date, and y^2 is the squared release date. To test whether the effect of length on recapture rate differed between the two categories of fish, the model above (H_A) was compared with a null hypothesis (H_0) that $\beta_2 = \beta_3$:

$$H_0: \text{Prob}_{(\text{recap})} = \alpha + \beta_1 d + \beta x + \beta_4 y + \beta_5 y^2$$

where x = fish length. The likelihood estimates of the respective models were used to form the likelihood ratio test statistic:

$$L_R = 2(\ln L_A - \ln L_0)$$

which is approximately χ^2 distributed ($df = 1$). For all statistical analysis, Systat version 5.0 (Wilkinson 1990) or Limdep version 5.1 (Green 1989) was used.

Results

Distributional Properties of Plasma Sodium Concentrations

The *p*-plot of plasma sodium data from immature fish revealed systematic changes of both location and shape of the

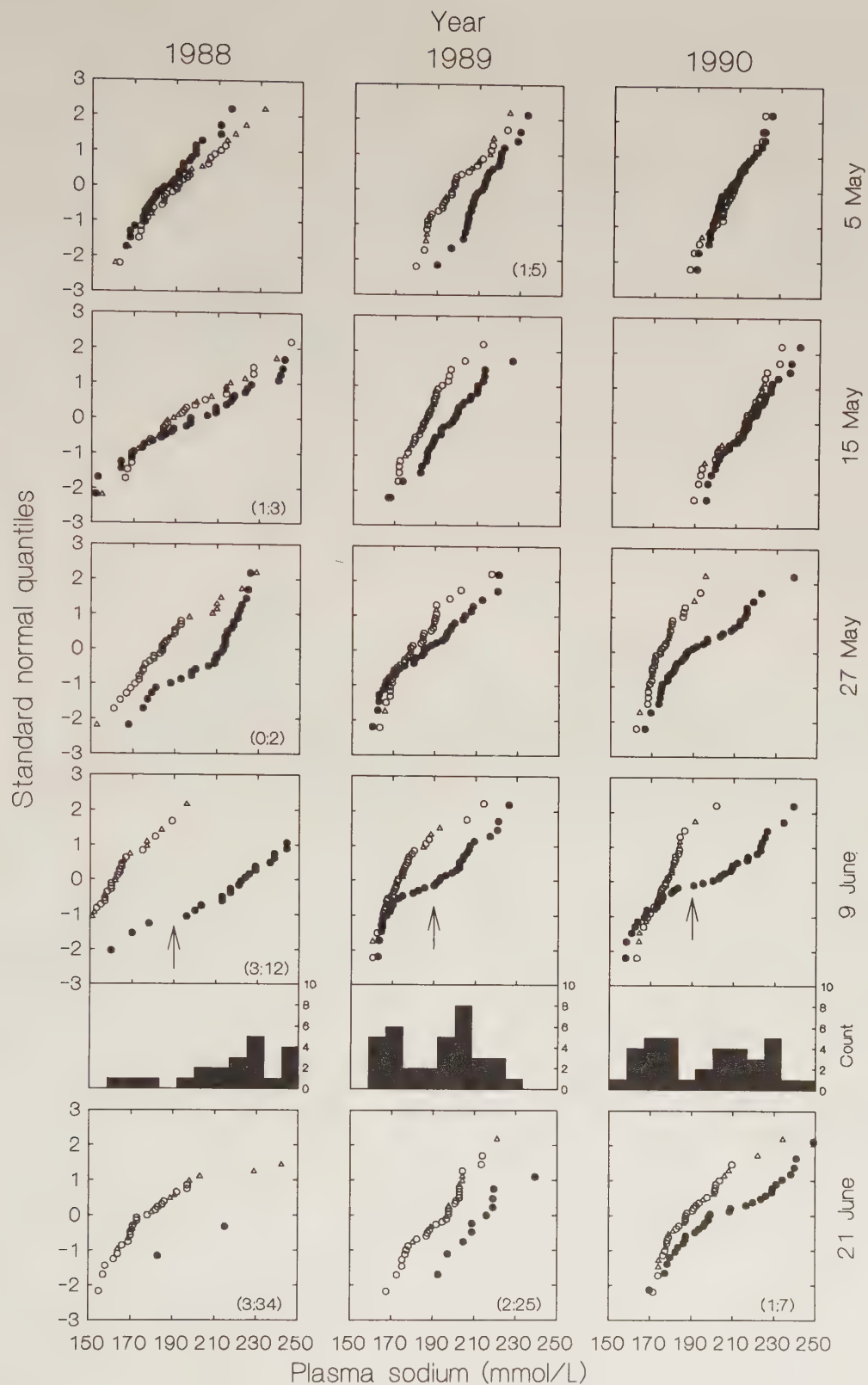


FIG. 2. Normal p -plots showing the distribution of blood plasma sodium concentrations in smolt-sized Atlantic salmon parr after a seawater challenge test at 25‰ during the smoltification period in three consecutive years. Data from previously mature males (solid circles) are superposed on data from previously immature fish (females = open circles; males = open triangles); $n = 36$ in each group. Sodium values from a normal distribution lie on a straight line. For each year the successive plots illustrate the development of a bimodal distribution (curved lines) of sodium values among males. Histograms for data from previously mature males are included at maximum hypoosmoregulatory ability. Arrows indicate the separation of fish with high (smolt-like) seawater adaptability from fish with low adaptability. Number of dead fish in the test is shown in parentheses (immature; mature).

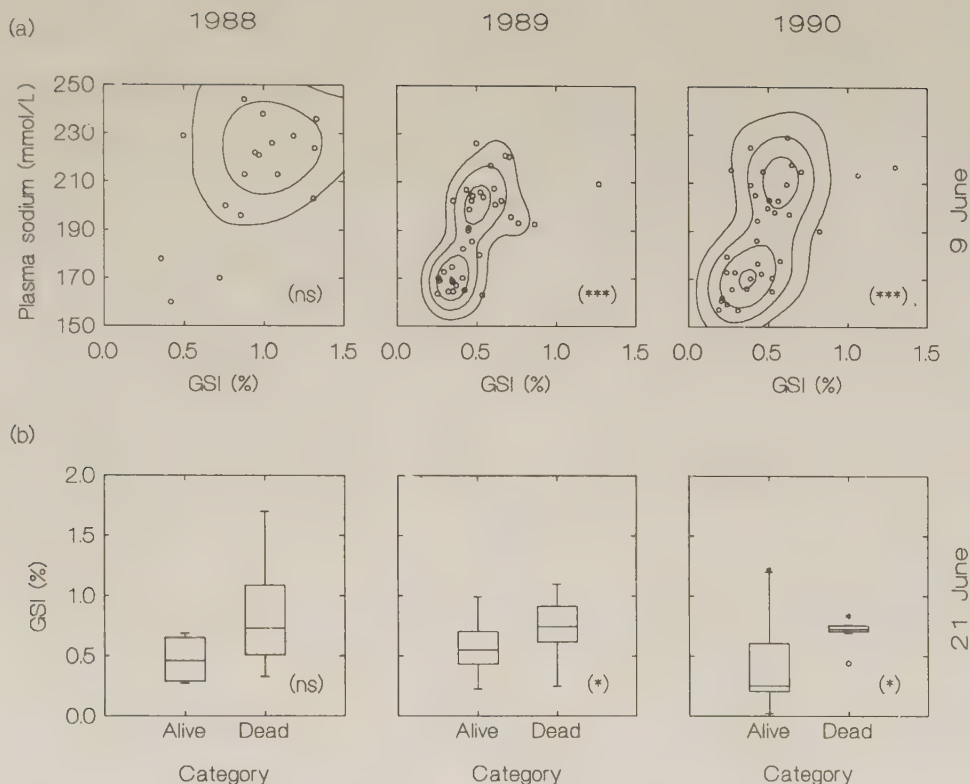


FIG. 3. (a) Bivariate scatter plot of plasma sodium values after seawater challenge versus GSI in previously mature males on 9 June. Isoclines of bivariate nonparametric kernel density estimators (Silverman 1986) are superposed on the data points and show areas with concentrations of data points. The distributions are bimodal in 1989 and 1990. ***Significant correlation ($p < 0.001$) by Spearman's rank correlation test. (b) Box-and-whisker plots of GSIs of previously mature males that died or stayed alive in the seawater challenge test on 21 June. * $p < 0.05$ by Mann-Whitney U -test.

distribution during the smoltification period in all years (Fig. 2). At the time of maximum hypoosmoregulatory ability, 27 May – 9 June, the plasma sodium concentrations of immature fish had become concentrated at the lower end of the scale. At that time the sodium concentrations also showed more narrow distributions compared with previous dates, as indicated by the steeper slopes of the p -plots. On 21 June the distributions of the plasma sodium concentrations were skewed towards higher values, indicating that part of the population had started to lose its hypoosmoregulatory ability.

The most striking feature of the frequency distributions of plasma sodium concentrations in previously mature males was the development of a bimodal pattern at smolting (Fig. 2). The bimodal pattern started to develop on 27 May each year. At the time of optimum seawater adaptability, some of the previously mature males formed a lower modal group with plasma sodium concentrations typical of immature fish (< 190 mmol/L), while other males formed an upper mode with higher sodium concentrations. In 1990, when the mortality at the last sampling date was low, the bimodal pattern was still distinguishable. In the other years, the proportion of mature males that remained alive after the last seawater challenge test was close to the proportion of males with low sodium values at the previous test date.

In 1988 and 1990 the plasma sodium concentrations of immature fish and previously mature males were similar until the bimodal distribution developed in mature males (t -test; $p > 0.05$). However, in 1989 the two categories differed on 5 and 15 May but not on 27 May. Thus, the hypoosmoregulatory

ability of immature fish and previously mature males was consistently different only after the male population had formed two groups. At the end of the smoltification period, a large number of previously mature males died in the challenge test, indicating an even poorer hypoosmoregulatory ability than that of the surviving males.

Except for a few deviating values, the plasma sodium concentrations of immature fish on 9 June were below 190 mmol/L in all years (Fig. 2). As the sodium values of previously mature males in the lower modal group also fell below 190 mmol/L, this value was used as a limit to separate fish with high seawater adaptability from fish with poor adaptability.

Correlation between Seawater Adaptability and Gonadal Development

During the smoltification period, a positive correlation between plasma sodium values and GSI developed. The correlation was significant ($p < 0.05$) from 27 May onwards in 1989 and 1990. Furthermore, there were two aggregations of data points in the bivariate (sodium versus GSI) distribution (Fig. 3a). One group of fish had relatively low sodium values and GSIs, and the other had comparatively higher values. In all years the GSIs of the males in the different sodium modes were significantly different by Mann-Whitney U -test ($p < 0.01$). The fish that died in the challenge tests had higher GSIs than fish that survived (Fig. 3b). Furthermore, the fish that died at the latest date not only had higher GSIs, but in most cases their gonads had changed external appearance from that

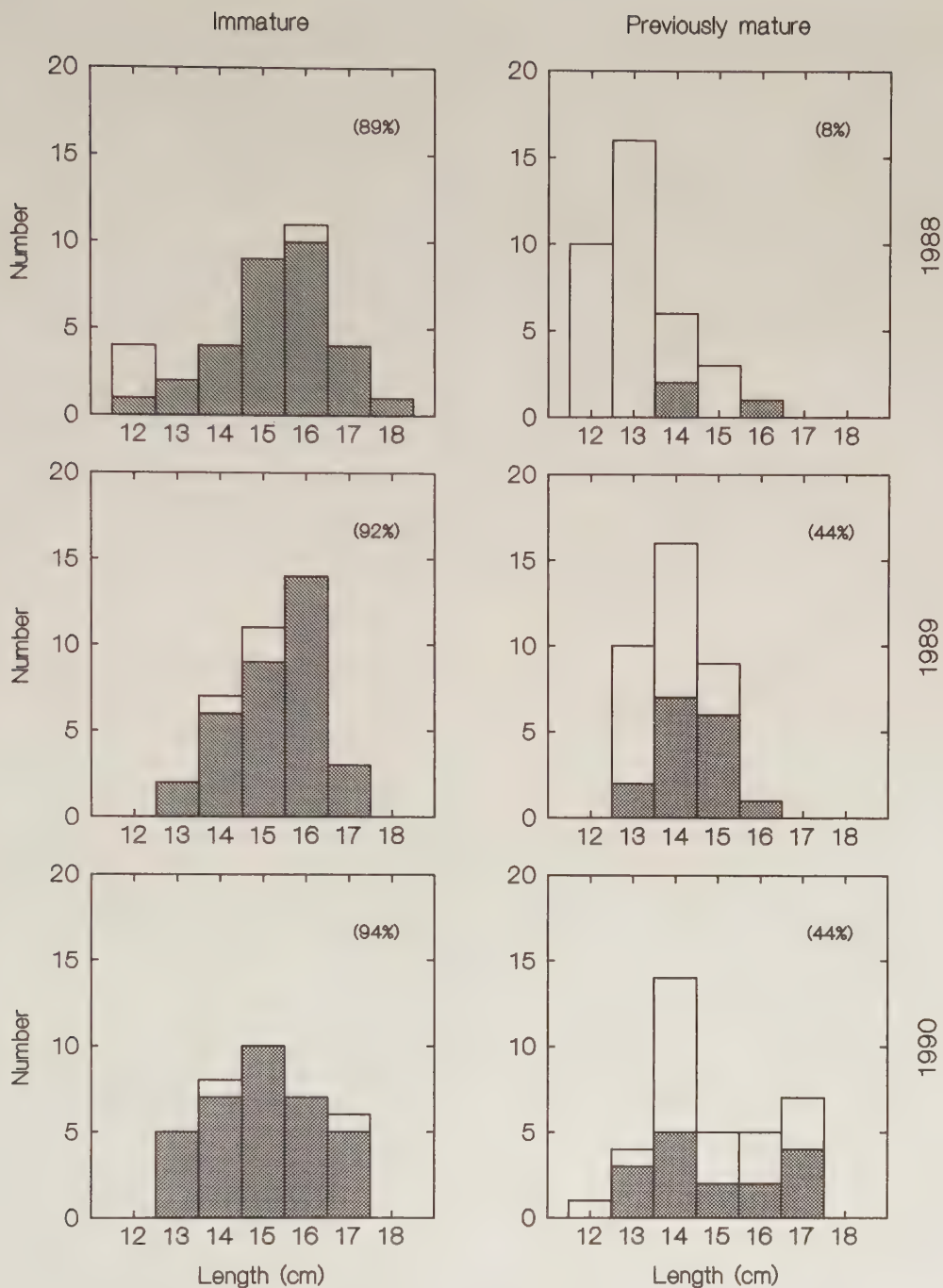


FIG. 4. Histograms showing the number of fish belonging to the groups with low (<190 mmol/L, i.e. smolting fish, cross-hatched bars) and high plasma sodium levels (open bars) after seawater challenge on 9 June for different length groups of immature and previously mature males during 1988–90. Total percent of fish with smolt-like seawater adaptability is shown in parentheses.

of gonads in a regressing stage to that of gonads in an early stage of maturation. Thus, the males with poor performance in seawater had started to mature again.

Effect of Size on Seawater Adaptability

The proportions of previously mature males with high seawater adaptability on 9 June were 8, 44, and 44% in 1988, 1989, and 1990, respectively (Fig. 4). In 1988 the previously mature males were particularly small, and the seawater adaptability was notably low among small males. In this year, all immature fish >12.5 cm developed high hypoosmoregulatory ability, while no previously mature males <14 cm ($n = 26$)

had high ability (Fig. 4). The proportion of previously mature males with high hypoosmoregulatory ability among fish >14 cm was on average 42%, and not different between years ($\chi^2 = 1.849$; $df = 2$; $p > 0.05$). However, this proportion was significantly lower than the proportions of well-adapted immature fish of similar size (on average 94%) in all years (Mantel-Haenszel $\chi^2 = 45.9$; $df = 1$; $p < 0.001$).

Seasonal Synchronization

In all three years, maximal seawater adaptability in females was found on 27 May or on 9 June, indicating a "smolt window" with high seawater adaptability from late May to mid-

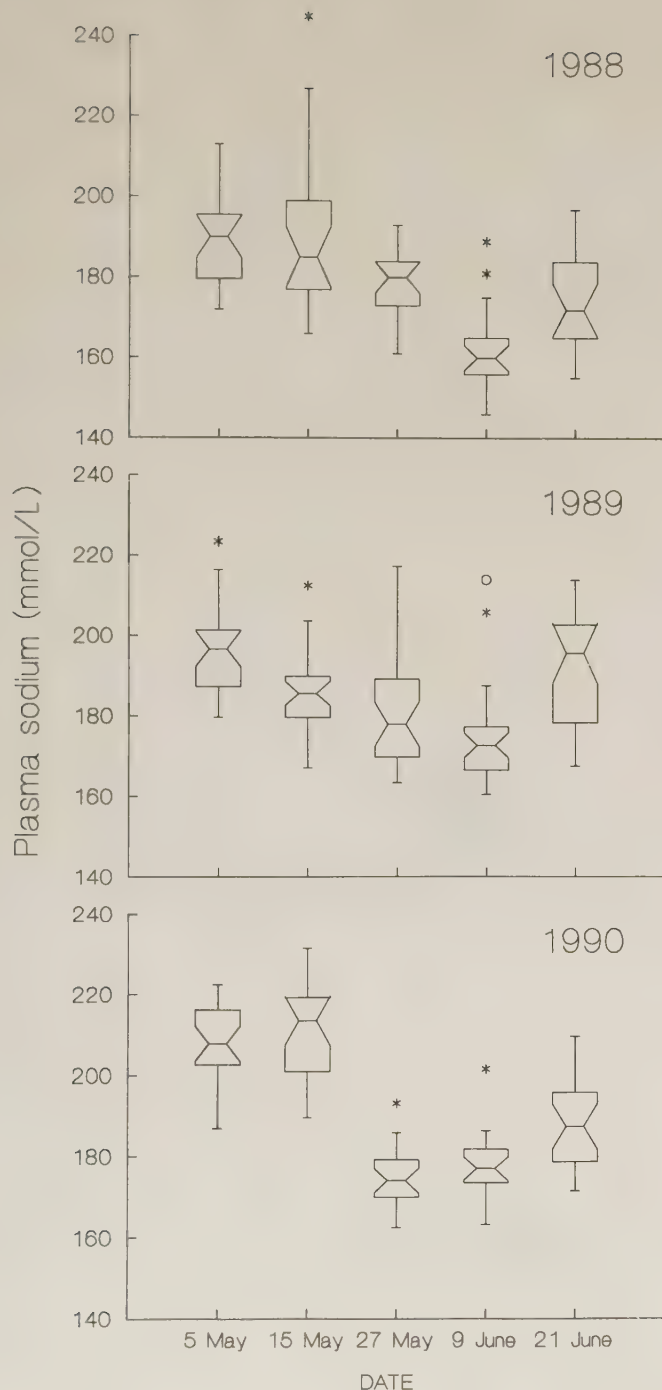


FIG. 5. Notched box-and-whisker plots of changes in plasma sodium concentration after seawater challenge in female smolts during the smoltification period during 1988–90. The notch corresponds to the width of the 95% C.I. about the median. The box gives the range of the middle 50% of the sodium values. The whiskers indicate the range of the sodium values, except for outliers (beyond 1.5 times the box height), which are plotted as individual points.

June (Fig. 5). The seasonal changes of plasma sodium concentrations after seawater exposure were significant (ANOVA; $p < 0.001$) in all years. In 1990, when the water temperature rose unusually quickly during spring (Fig. 1), the hypoosmoregulatory ability developed earlier than in the other years. Apart from the low sodium values, the date with the highest hypoosmoregulatory ability was also characterized by a narrow and symmetrical distribution of sodium values.

Adult Recapture Rates

There was a pronounced variation in the recapture rate of released fish due to life history type, size at tagging, and release date. The total recapture rate of released immature fish was about 7 times higher than that of previously mature males. In both groups the seasonal changes in recapture rate were similar, with highest recapture rate for fish released on 27 May (Table 1).

Using a probit model (likelihood ratio of fit, $\chi^2 = 849.34$; $df = 5$; $p < 0.001$), life history type (immature and mature), fish length, release date, and squared release date were all found to affect the adult recapture rate significantly (Table 2). In addition, the effect of length on recapture rate was different for the two life history types (likelihood ratio test, $\chi^2 = 25$; $df = 1$; $p < 0.001$). The recapture rates predicted for previously mature males were particularly low for small fish and increased faster than for immature fish in the upper half of the length distribution (Fig. 6). Previously mature males with a size of 17 cm had about 9 times higher observed total recapture rate than males in the 14-cm group (Table 1). The corresponding increase in the recapture rate with respect to size was only about 2 times for immature fish.

Correlation between Seawater Adaptability and Recapture Rates

The patterns of seasonal changes in seawater adaptability and recapture rate for different release dates were similar (Fig. 7). The recapture rate was highest for fish released about 2 wk before the time of optimal seawater adaptability. When the latter curve was shifted minus 13 d, so that optimal recapture rate and optimal seawater adaptability coincided, a high correlation was found between the two variables ($R^2 = 0.922$ and $R^2 = 0.955$ for recaptures of immature and previously mature fish, respectively).

Discussion

An important finding of this study was that smolt-sized previously mature male parr fell into two groups at the time of optimal seawater adaptability. One of these groups developed a seawater adaptability similar to that of immature males and females, while the other did not. Furthermore, males in the group with poor hypoosmoregulatory ability had higher GSI than the fish in the smolting group. In all three years, there was a pronounced seasonal variation in seawater adaptability, with a peak in adaptability in early June. This physiological smolting pattern was reflected in the recapture rate of adults for all release dates.

At the time of maximum seawater adaptability the plasma sodium concentrations were not only low, but also characterized by a narrow and symmetrical distribution. This indicates a qualitative change of the hypoosmoregulatory ability in smolting salmonids which is distinct from the size-dependent changes in salinity tolerance found in nonsmolting fish (cf. McCormick and Saunders 1987). The rather narrow smolting period found in the present study is further supported by earlier studies on the same stock (Lundqvist and Eriksson 1985; Lundqvist et al. 1989). The temperature during spring months has been found to affect the development of hypoosmoregulatory ability in smolting fish (Wagner 1974; Zaugg and McLain 1976; Saunders et al. 1982; Jonsson and Ruud-Hansen 1985). This might explain the earlier occurrence of maximal seawater adaptability in 1990.

TABLE 1. Number of fish released and percent recaptured (in parentheses) in relation to release date and size at release.

Category and size group (cm)	Date of release					
	5 May	15 May	27 May	9 June	21 June	Total for group
Previously mature						
13.0	428 (0.0)	468 (0.0)	396 (0.2)	457 (0.9)	447 (0.2)	2196 (0.3)
14.0	614 (0.2)	619 (0.5)	620 (1.1)	653 (0.6)	590 (0.7)	3096 (0.6)
15.0	555 (0.7)	527 (2.5)	557 (2.7)	517 (1.4)	570 (2.1)	2726 (1.9)
16.0	290 (2.4)	270 (4.4)	297 (7.7)	264 (4.6)	285 (1.4)	1406 (4.1)
17.0	94 (4.3)	89 (11.2)	103 (5.8)	81 (3.7)	77 (2.6)	444 (5.6)
>18.0 ^a	8	6	9	7	19	49 (6.1)
Total for date	1989 (0.8)	1979 (1.9)	1982 (2.7)	1979 (1.6)	1988 (1.2)	9917 (1.6)
Immature						
13.0	30 (3.3)	38 (0.0)	49 (8.2)	56 (5.4)	43 (4.6)	216 (4.6)
14.0	97 (3.1)	106 (11.3)	98 (12.2)	104 (4.8)	97 (2.1)	502 (6.8)
15.0	226 (8.0)	252 (12.3)	234 (14.5)	214 (8.4)	212 (9.4)	1138 (10.6)
16.0	310 (8.7)	263 (13.7)	288 (21.2)	278 (15.8)	308 (12.0)	1447 (14.2)
17.0	237 (9.3)	227 (11.9)	212 (18.9)	224 (14.7)	238 (10.5)	1138 (12.9)
>18.0	97 (12.4)	113 (17.7)	107 (16.8)	109 (13.8)	98 (11.2)	524 (14.5)
Total for date	997 (8.3)	999 (12.6)	988 (17.1)	985 (12.0)	996 (9.7)	4965 (11.6)

^aRecapture rates all <1%.

TABLE 2. Probit analysis of recapture data.

Maximum likelihood estimates		Variable	Coefficient	SE	T-ratio	p
Log-likelihood	-2298.6	Constant	-21.39	1.952	-10.9	<0.001
Restricted (slopes = 0)						
log-likelihood	-2723.2	d (state of maturity)	3.78	0.614	6.1	<0.001
χ^2 (df = 5)	849.34	x_1 (d-length immature)	0.14	0.024	6.1	<0.001
Significance level	<<0.001	x_2 (d-length previously mature)	0.34	0.031	10.8	<0.001
		y (release date)	0.19	0.026	7.4	<0.001
		y^2 (squared release date)	-0.64×10^{-3}	0.860×10^{-4}	-7.4	<0.001

We found that optimal recapture rate occurred when fish were released before the time of maximum seawater adaptability. This indicates that migratory behaviour starts to develop earlier than hypoosmoregulatory ability, as found by Lundqvist and Eriksson (1985). This is also consistent with the conclusion by Ewing et al. (1980) that changes in gill Na^+ , K^+ -ATPase activity relate more closely to periods of seawater entry than to periods of seaward migration in long rivers.

In the present study, it was found that previously mature males with poor hypoosmoregulatory ability had higher GSIs than those with high ability. Furthermore, their gonads often had started to grow by the end of the smoltification period. This suggests that the reason for the bimodal smoltification pattern among these males is that some males start to mature again by early June and therefore do not develop high seawater adaptability. A similar pattern was found when we reanalysed data from this stock collected in 1986 and 1987 (Lundqvist et al. 1990; L.-O. Eriksson and T. Eriksson, unpubl. data). Our conclusion is also consistent with recent results by H. Fängstam, I. Berglund, M. Sjöberg, and H. Lundqvist (unpubl. data) on the downstream migratory behaviour of smolts from the same population. They found that previously mature males with growing gonads by the end of June did not show the migratory behaviour that was prevalent in males with undeveloped gonads at that time. In addition, Berglund (1991) recently demonstrated that androgen treatment prevented migratory behaviour in smolt-sized immature male and female Baltic salmon. These findings correspond well with small elevations of plasma

androgens in May-June that have been observed in previously mature male parr from the same stock (Mayer et al. 1990b).

Plasma androgens in mature parr reach low levels soon after the spawning period is over and do not rise until maturation starts in May-June the following spring (Mayer et al. 1990b). However, differences in androgen metabolism in the brain between immature and previously mature fish prevail in April several months after spawning (Mayer et al. 1991). Castration of mature male parr during the breeding season lowers the androgen levels drastically (Mayer et al. 1990a). Although androgens impair seawater adaptability in several salmonids (Ikuta et al. 1985; Miwa and Inui 1986; Lundqvist et al. 1989), springtime castration does not restore the hypoosmoregulatory ability in previously mature male Atlantic salmon parr (Lundqvist et al. 1990). A possible explanation for the absence of a positive effect of castration on seawater adaptability might be that the osmoregulatory system is affected by androgens of extragonadal origin. Steroidogenic capacity has been found in the head kidney (Sangalang and Freeman 1988), which is a site of testosterone production in spring in masu salmon (*Oncorhynchus masou*) (Yamada et al. 1988). The early elevation of androgen levels found in maturing male parr at the end of May (Mayer et al. 1990b) might be of extragonadal origin.

Our results suggest that only about half the previously mature males develop high hypoosmoregulatory ability comparable with that in immature fish. However, in some years the effect of size seems to be stronger in previously mature males, resulting in very low smolting rate in small smolt-sized fish. The

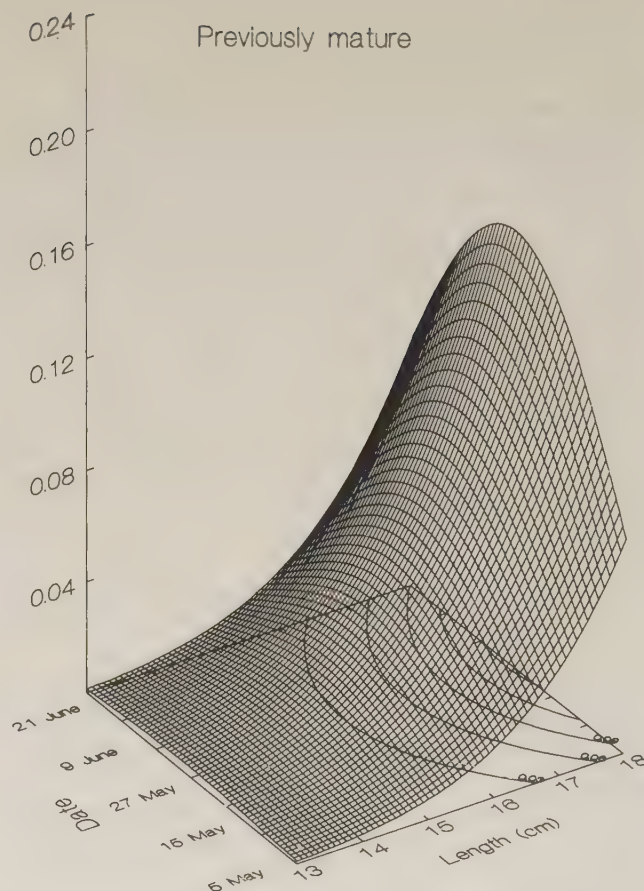
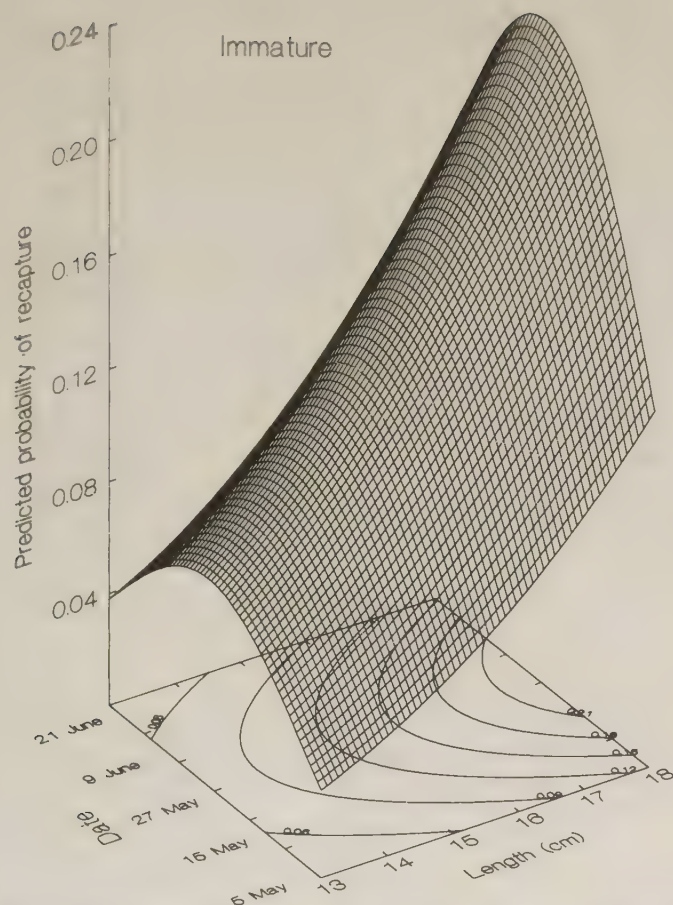


FIG. 6. Predicted probabilities of adult recapture rates for releases from immature fish and previously mature males in relation to fish size and release date in 1988. Probabilities obtained by probit regression using data on length at tagging and adult recapture incidence of individual fish. The regression equations are $\text{Prob}_{(\text{recap})} = \text{ZCF}(0.144(\text{length}) + 0.191(\text{date}) - 0.640 \times 10^{-3}(\text{date})^2 - 17.6)$ and $\text{Prob}_{(\text{recap})} = \text{ZCF}(0.339(\text{length}) + 0.191(\text{date}) - 0.640 \times 10^{-3}(\text{date})^2 - 21.4)$ for immature fish and previously mature males, respectively. ZCF = the normal cumulative distribution function.

finding that larger fish had a higher probability of recapture is in agreement with previous findings by Carlin (1955, 1959), Larsson (1984), and Lundqvist et al. (1988). Interestingly, the effect of size on recapture rate was much more pronounced among previously mature males than among immature fish. It seems reasonable to assume that this difference was due to the low smolting rate among small previously mature males in 1988 (see Fig. 4 and 6). Lundqvist et al. (1988) found a lower recapture rate from releases of previously mature males compared with immature fish and related it to incomplete smolting in mature males. The bimodal smoltification pattern found in the present study supports this suggestion.

In 1988, there were no smolting fish among previously mature males in the 13-cm group, while smolting fish were present in this size group the following years. This, suggests that factors other than size might be important. The possibility that winter and spring temperatures may affect the decision of a male whether to smoltify or mature again depends on when the decision is taken. Although androgens interfere with both physiological and behavioural aspects of smolting in spring, as shown in different experiments with androgen treatment, it is possible that the presence or absence of high androgen levels in winter-spring is not the primary reason for the lack of hypoosmoregulatory ability in some previously mature males; the "decision" to smoltify or mature again may have been made already. In many North American, Scottish, and Norwegian

salmon populations (Thorpe 1977; Bailey et al. 1980; Skilbrei 1988) the fish with the fastest growth rate during the first summer form an upper modal group that will smolt at age 1+. Thus, the smoltification process seems to start in the summer-autumn and is completed the following spring. In the population studied in this paper, smoltification at age 1 is not possible due to the poor growth during the first summer. Instead, males that grow large during the first summer tend to mature sexually the second summer (Berglund 1992). Since the maturing males grow more slowly the second summer (Berglund 1992; Berglund et al. 1992), they will be smaller at the end of their second summer. In most salmon populations the size threshold for smoltification appears to be 12–13 cm (Wedemeyer et al. 1980); therefore, males that mature may fall below the threshold for smolting (Bailey et al. 1980). In addition, the present findings on seawater adaptability and recapture rates suggest that the size threshold for smolting, at least in some years, may be higher for previously mature males. The probit model fitted to recapture data found that the increase in recapture rate with respect to length was larger for previously mature males than for immature fish. Allowing for extrapolation outside the range of data, the model predicts that large previously mature males (19.5 cm) should have the same recapture rate as immature fish of similar size. Thus, large previously mature males might smoltify in the same numbers as immature fish. This suggests that a conditional mechanism involving body size as an impor-

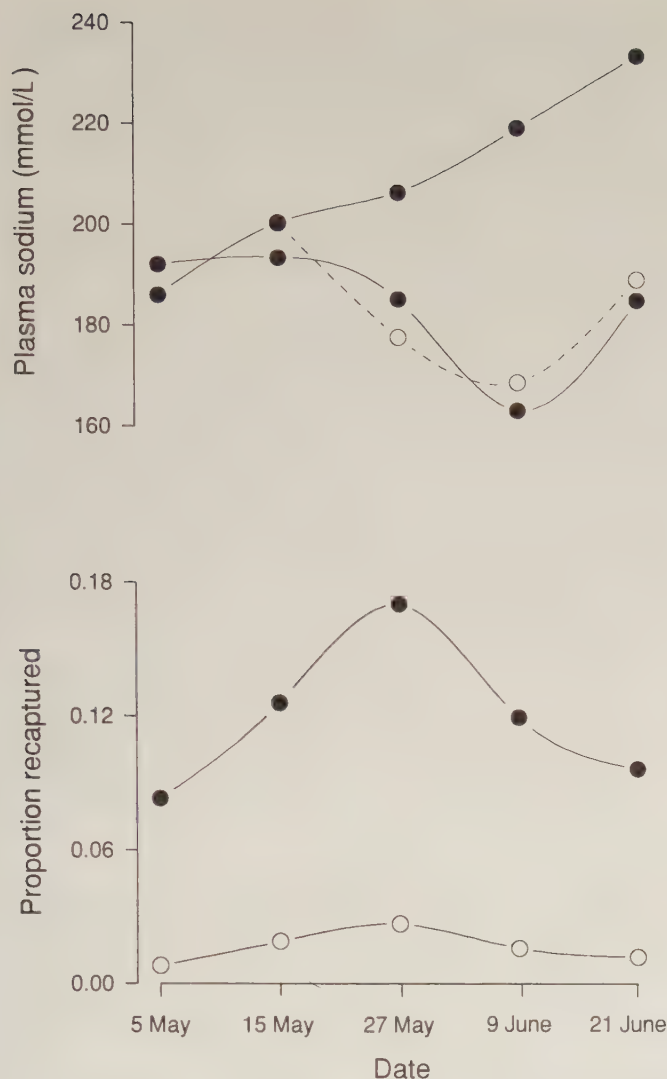


FIG. 7. Mean plasma sodium concentrations of previously immature fish and observed recapture rates in the sea fishery (immature fish = solid circles; previously mature males = open circles) as functions of sampling date or release date, respectively. The few males that formed the lower modal group with plasma sodium concentrations typical of immature smolting fish in 1988 (cf. Fig. 2) are shown separately with the broken line.

tant factor influences the choice of life history in previously mature male parr. However, from the results in 1989 and 1990, it is clear that a relatively large size (14–18 cm) is not enough to ensure smolting in previously mature males reared at low temperature.

Berglund et al. (1991a) found that previously mature males kept at elevated water temperature (9°C) the whole winter had much higher hypoosmoregulatory ability than control fish at the time of normal smolting, and a lower proportion of males reared in heated water matured again during their first summer in seapens. Rearing in heated water also enhanced the number of downstream migrating males released in a river in spring to a level similar to that of immature fish. Fish kept in heated water over winter increased their length and weight and most likely also their lipid levels. Saunders et al. (1982) found that previously mature males reared at 10°C increased their lipid levels from December to March. Thus, the improved performance of heated fish may be due to both increased size and increased energy stores that may be required for successful smoltifica-

tion, stores that had been depleted during sexual maturation in the autumn.

It might be questioned if Baltic salmon need to develop a hypoosmoregulatory ability to the same extent as salmon migrating to more saline environments. However, the present study suggests that seawater adaptability in Baltic salmon is not only linked to changes in condition factor and external appearance (Lundqvist 1983), but also that the seasonal changes in hypoosmoregulatory ability are temporally related to some other aspects of smolting that may affect survival and recapture rate. Therefore, hypoosmoregulatory ability may serve as a good predictor for the probability of successful seaward migration and high postsmolt survival. The importance of time and size at release of coho salmon (*Oncorhynchus kisutch*) has been stressed by Bilton et al. (1982).

The proportion of mature male parr may reach 50% in many Baltic salmon stocks (Lundqvist et al. 1988). Together with the large variation in the proportion of smolting by previously mature males (8–44%) that was found in the present study, this implies a great impact on the population structure of sea-run salmon. As early sexual maturation does not seem to affect recapture rate in more southern stocks (Hansen 1980), and the proportion of smolting males may be increased if the fish are kept at elevated winter temperatures (Berglund et al. 1991a), the proportion of smolts among previously mature males is likely to have a north–south gradient.

It appears that part of the population of previously mature males studied in this paper matures sexually again in spring and that the decision of an individual male is affected by body size and the conditions for growth during the previous autumn–winter. The reason why some previously mature males do not smoltify, although they seem large enough, may be energetic constraints. If so, to mature again may be the best choice in a bad situation. Alternatively, male parr that grow too large may have low prospective success as sneakers at the spawning ground and therefore, as a result of disruptive selection (Gross 1985), become smolts and migrate seawards.

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Relationship between Annual Recruitment and Density in a Lacustrine Population of Allopatric Brown Trout (*Salmo trutta*)

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Borgstrøm, R. 1992. Relationship between annual recruitment and density in a lacustrine population of allopatric brown trout (*Salmo trutta*). Can. J. Fish. Aquat. Sci. 49: 1107–1113.

The annual recruitment of brown trout (*Salmo trutta*) parr from stream nursery areas to an allopatric, lake-resident population was studied during a 5-yr period. The immigration of each cohort to the lake occurred over several years, but the data indicate that the duration of the lotic residence of parr may be regulated by the density of the lake population. At high lake population density, the number of brown trout in age-class 3+ present in the lake by June was low, while at low lake population density the number of 3+ fish increased substantially.

Une étude d'une durée de 5 ans a été effectuée sur le recrutement annuel, dans une population lacustre allopatrique, de jeunes truites brunes (*Salmo trutta*) au stade de tacon, provenant des aires d'alevinage de divers cours d'eau. La migration de chaque cohorte dans le lac s'est étalée sur plusieurs années, mais les données indiquent que la durée de résidence lotique des jeunes truites brunes peut être tributaire de la densité de la population lacustre. D'une part, lorsqu'il y avait une forte densité de la population lacustre, on a enregistré un petit nombre de truites brunes âgées de 3 ans et plus dans le lac en juin; d'autre part, lorsque la densité de la population lacustre était faible, le nombre de poissons de la classe d'âge 3+ a augmenté considérablement.

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In populations of wild brown trout (*Salmo trutta*), high densities commonly result in small, slow-growing fish (Sømme 1941; Campbell 1967, 1971). Sometimes, high fishing pressure can substantially reduce the number of old fish and may result in increased growth rates of the remaining population (Jensen 1977). At other times the total number of fish is not reduced by fishing (Dahl 1943), possibly due to an increase in number of immigrants from nursery areas. In stream-spawning populations of lake-resident brown trout, the youngest age-classes stay in lotic habitats up to several years before they migrate into the lake (Jonsson 1985, 1989). Changes in duration of lotic residence in response to changes in density and structure of the population residing in the lake could explain stable population size despite increased fishing. In the work described here, I investigated the relationship between population number of brown trout in a small Norwegian lake and the age and number of recruits from the nursery streams.

Methods

Study Area

Lake Løyning is situated in Western Norway, 595 m above sea level (Fig. 1), in the subalpine region. The surface area is 0.49 km², maximum depth approximately 45 m, and mean depth 14.7 m. The lake is oligotrophic, with summer Secchi disc transparencies in the range 10–14 m. Ice breakup usually takes place by the end of May. Brown trout is the only fish species present in the lake. Piscivory has not been found after examination of more than 4000 brown trout stomachs. The main diet consists of chironomids, small crustaceans, and terrestrial insects. Mink (*Mustela vison*) are regularly observed along the main river where heron (*Ardea cinerea*) are occasionally seen as well. Loons and mergansers have not been observed, and predation upon the brown trout in the lake must be insignificant.

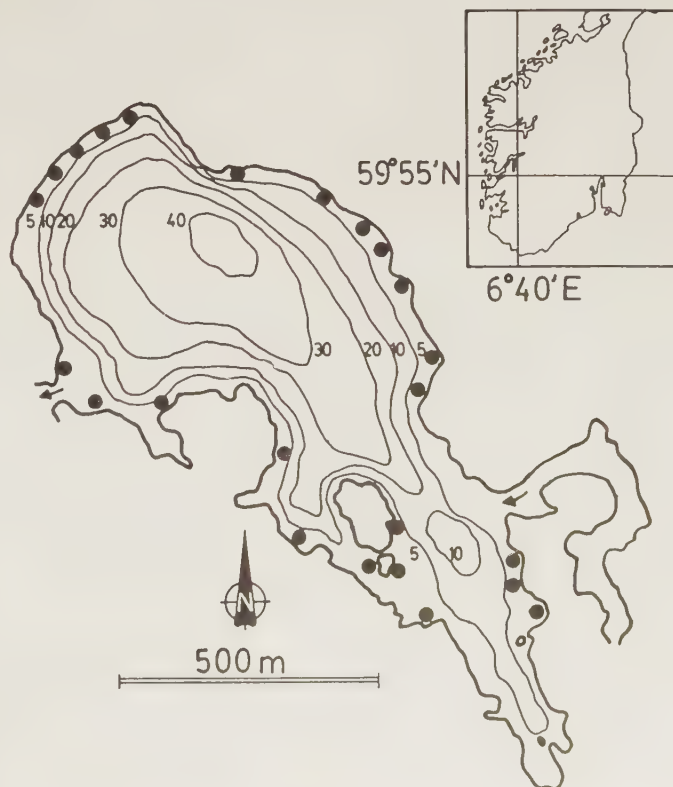


FIG. 1. Geographical location and contour map of Lake Løyning. Solid circles indicate sampling sites for beach seining.

TABLE 1. Petersen estimates of number of brown trout present in Lake Løyning in June 1985–89 based on number of recaptures in year of marking after back-calculation of length at capture to length at marking.

Year	Length-class (cm)	Number marked first fishing (M)	Number caught second fishing (C)	Recaptures (R)	Estimated number (N)	Confidence limits (0.95)
1985	14.0–25.9	785	1268	97	10 178	8359–12 388
	14.0–16.9	138	135	14	1 260	772–2173
	17.0–19.9	320	363	40	2 850	2105–3947
	20.0–21.9	211	376	30	2 578	1825–3770
	22.0–23.9	102	323	12	2 567	1517–4635
	24.0–25.9	14	71	1	—	—
1986	14.0–25.9	592	863	57	8 834	6845–11 387
	14.0–16.9	55	213	6	1 712	850–3745
	17.0–19.9	185	233	24	1 741	1189–2654
	20.0–21.9	180	157	15	1 787	1108–3042
	22.0–23.9	131	198	11	2 189	1269–4104
	24.0–25.9	31	62	1	—	—
1987	14.0–25.9	479	774	74	4 960	3962–6206
	14.0–16.9	70	200	10	1 297	736–2504
	17.0–19.9	146	181	25	1 029	708–1555
	20.0–21.9	129	168	19	1 099	718–1758
	22.0–23.9	98	170	15	1 058	656–1801
	24.0–25.9	36	55	5	345	163–797
1988	14.0–25.9	356	361	35	5 772	4182–7942
	9.0–13.9	209	149	8	3 500	1875–7159
	14.0–16.9	139	211	14	1 979	1211–3411
	17.0–19.9	125	150	14	1 268	777–2187
	20.0–21.9	53	81	4	886	395–2214
	22.0–23.9	28	89	3	653	266–1631
1989	14.0–25.9	776	830	75	8 496	6796–10 614
	14.0–16.9	235	263	22	2 709	1822–4210
	17.0–19.9	371	307	35	3 183	2305–4529
	20.0–21.9	104	148	14	1 043	639–1798
	22.0–23.9	43	90	4	801	358–2002

The brown trout spawn in the river above and below the lake and in a few small inlet streams. During September, fishermen use gill nets to catch a high number of the mature fish as they ascend the inlet river. No sampling of parr in the streams was carried out in the present study because of the high discharge and the low conductivity of the water (around $10 \mu\text{S}\cdot\text{cm}^{-1}$), resulting in extremely low electrofishing efficiency. Lake spawning has never been recorded.

Few brown trout obtain lengths above 25 cm in this lake, although a few years after being stocked into lakes devoid of fish, brown trout from Lake Løyning obtain lengths around 40 cm.

The lake is open both for sportfishing and gillnetting. The gill nets used by the ordinary fishery have mesh sizes around 26 mm (bar measure), catching only the upper length-classes of the population. Because of this, the exploitation of the population as a whole was low for several decades, and at least during the last 30 yr, there was a dense population of stunted brown trout in the lake. Fishing mortality was increased considerably by my experimental gillnetting in 1985–89 and by the beach seining carried out in August–September 1985–87 by residents. From 1985 to 1988, about 2000 fish were captured

annually by this increased fishing, in addition to the catch obtained by the ordinary sport- and gillnet fishery.

Population Estimation

The number of brown trout in the lake was estimated each year by the mark–recapture method (Petersen method). During June 10–20, brown trout were captured for marking by a 50-m-long beach seine with 5-mm mesh size (bar measure), and the population estimates refer to this period. The fine-meshed beach seine retained fish with lengths above 6–7 cm. The beach seine sampling was distributed around the lake shore wherever it was possible (Fig. 1). The captured fish were anaesthetized, total length measured to the nearest millimetre, and subsequently marked and released at the capture site. The fish were marked either by removal of the adipose fin or by inoculation of Alcian Blue at the base of the pectoral, ventral, anal or caudal fins by a jet inoculator (Hart and Pitcher 1965; Pitcher and Kennedy 1977). By these methods, each year's marking could be separately coded. In 1986, each centimetre-class was given a specific marking code with the Alcian Blue to test the back-calculation from length at capture to length at marking. To check loss of marks by the Alcian Blue method,

half of the adipose fin was removed in 1986. No recovered fish with half an adipose fin had lost the blue marks, and loss of marks is therefore considered to be insignificant.

Random sampling for capture of marked fish was carried out by a combination of gill nets set uniformly on the bottom in the littoral zone, and gill nets set at least 50 m from the shore, in the depth intervals 0–6 and 6–12 m. The netting sites were used several times each year, but with different mesh sizes. The length of all gill nets was 25 m, with a height of 1.5 m for littoral nets and 6.0 m for pelagic nets. Both littoral and pelagic gill nets had mesh sizes of 16, 19.5, 21.0, 22.5, 24.0, and 26.0 mm (bar measure). The largest mesh sizes of this gillnet gang efficiently capture brown trout with lengths in the range 22–30 cm (Jensen 1977). Gillnetting commenced when the marking was finished and lasted until late August (1985–88) or September 3 (1989).

All fish caught by the experimental gill nets were checked for marks, weighed to the nearest gram, and total length measured to the nearest millimetre. Scales and otoliths were taken for age determination and back-calculation of length. The otoliths were cut in half through the nucleus and burnt before reading (Power 1978).

A number of conditions, listed by Ricker (1975), must be met in population estimation by mark–recapture. Marked and unmarked fish must have the same mortality. If mortality due to handling and marking varies with fish size, then a bias occurs in estimated numbers of different size-classes. However, a comparison between the length distribution of marked fish and the length distribution of recaptures in year of marking or the following year did not indicate any variation in mortality. The chosen marking methods are not expected to change the vulnerability to gillnetting because no tags were used.

To avoid bias due to recruitment into a length-class and loss of fish from a length-class due to growth, the lengths of all captured fish were back-calculated to time of marking (start of growing season) by the method of Lea–Dahl (Lea 1910; Dahl 1910). When an otolith showed a higher number of annuli than the scale, this was taken as an indication of cessation of growth in length (Power 1978), and no back-calculation was done. The back-calculation method was controlled by using the recaptured marked fish from 1986. Obtained lengths after back-calculation from length at capture were all within the centimetre-class the fish was marked in, or within 1–3 mm from the expected length-class, indicating the adequacy of the method. The validity of the age determination by otoliths was checked by using recaptured fish from Lake Løysning stocked into a lake devoid of fish. The distance between the annuli on otoliths from these fish reflected the increased growth rate after stocking, and the number of annuli agreed with the number of years between stocking and recapture.

In all years, adjusted Petersen estimates (Ricker 1975) were obtained for size-classes above 14.0 cm according to the equation

$$N = \frac{(M + 1)(C + 1)}{(R + 1)}$$

where N = estimated number in population, M = number of marked fish, C = number captured and controlled for marks, and R = number of recaptures. Confidence limits for the estimates were obtained according to Ricker (1975). I assumed that fish within short length intervals had approximately equal capture probabilities by beach seining. Thus, the number of fish within each separate size-class (14.0–16.9, 17.0–19.9, 20.0–21.9, 22.0–23.9, and 24.0–25.9 cm) estimated by the mark–

recapture method may be divided into centimetre-classes in proportion to the number captured by the beach seine.

Fish in the size-class 9.0–13.9 cm were also marked every year, but the number of recaptures in the year of marking was too low for an estimate of number of fish within this size-class by the mark–recapture method, except in 1988. In addition, immigration of young fish between marking and recapture may give an overestimation by the mark–recapture method, as indicated by the result from 1988. By the mark–recapture method, the estimated number of fish in size-class 9.0–13.9 cm was 3500 in June 1988, while the number in beach seine catches corresponded to 2976 fish by the proportional method. Therefore, estimates for each centimetre-class in the size-class 9.0–13.9 cm were obtained for all years by assuming equal catchability by beach seine for fish in this size-class and size-class 14.0–16.9 cm. Likewise, the number of fish in size-class 24.0–26.9 cm was estimated by the proportional number in beach seine catches for this length-class and length-class 22.0–23.9 cm.

To calculate the total number of fish within each year-class, the number of fish within each centimetre-class was apportioned to year-classes according to the frequency of each year-class within the centimetre-class, and the obtained numbers were summed for all centimetre-classes.

Results

Population Numbers and Length Distribution

Both gillnet and beach seine catches indicated that the population mainly consisted of fish in the length range 9–26 cm (Fig. 2). During the whole study, only seven brown trout with lengths 27 cm or greater were captured by beach seine and gill nets. The number of brown trout below 9 cm in length (ages 1+ and 2+) captured by beach seine in June was less than 10 each year, except in 1988 when 41 were caught.

By the Petersen method, the estimated number of fish 14.0 cm or greater in length ranged from 5000 to 10 000 (Table 1). By estimating the number of fish within each centimetre-class in the length range 9–26 cm, the resulting length distributions of the population show a change from a dominance of fish above 20 cm in length in June 1985–86 to a dominance of fish with lengths below 15 cm in June 1988, with a displacement towards larger fish in June 1989 (Fig. 3).

In June, age 3+ brown trout had back-calculated lengths in the range 10–19 cm, with small annual variations in mean lengths and variances (Fig. 4). The mean back-calculated length for only one year (1987) was significantly greater (t -test, $p < 0.05$) than the others. Therefore, the annual variation in captured and estimated number of brown trout in the length range 10–15 cm was mainly due to variation in occurrence of age 3+ brown trout and not to differences in beach seine vulnerability of age 3+ brown trout caused by change in growth rate and size of fish in this age-class.

Population Density and Recruitment

Within each year-class, the numbers of age 3+ fish present in the lake by June were generally lower than the numbers of age 4+ fish by June in the following year, and the immigration of a cohort to the lake probably was not completed before age 5+ (Table 2). The number of age 3+ fish (R) in the lake population was negatively related to the number of fully recruited year-classes (age 5+ and older fish) (N) ($\ln R = 8.597 - 0.00037N$; $p < 0.005$, $r^2 = 0.9683$) (Fig. 5). The same rela-

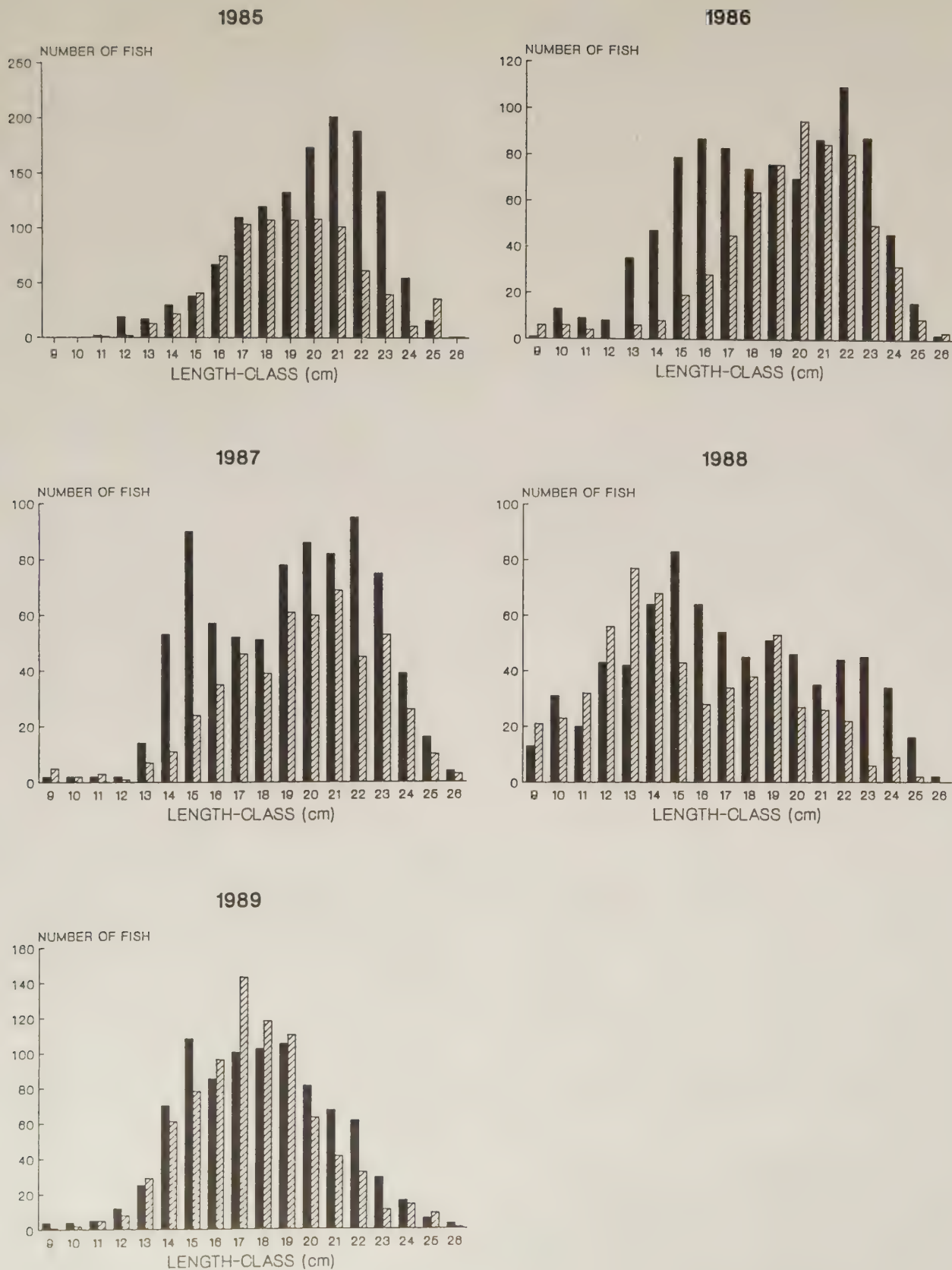
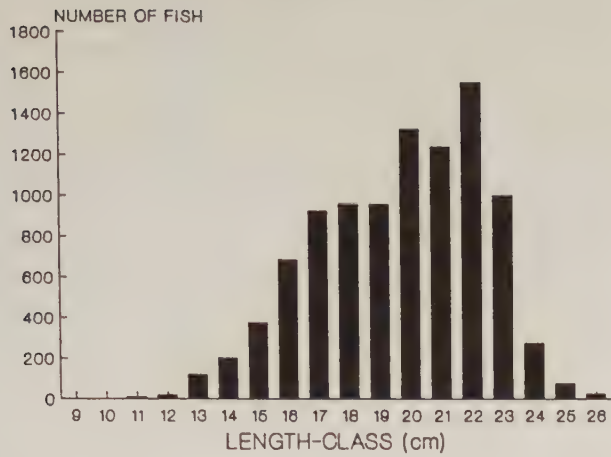


FIG. 2. Length-frequency distribution of marked brown trout sampled by beach seine in Lake Løyning, June 1985–89 (hatched bars), and length-frequency distribution of brown trout captured by gill nets and examined for marks back-calculated to length in June (solid bars).

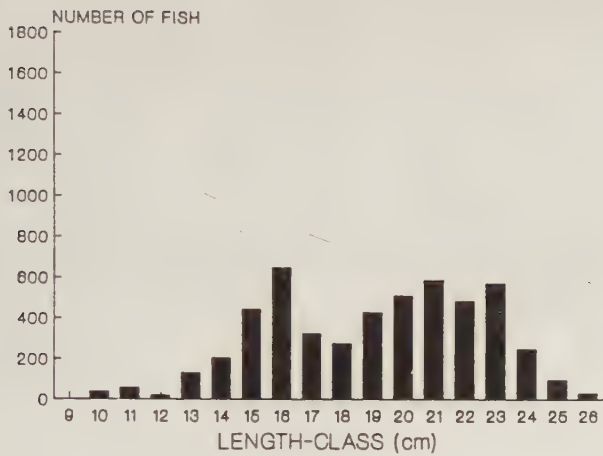
1985



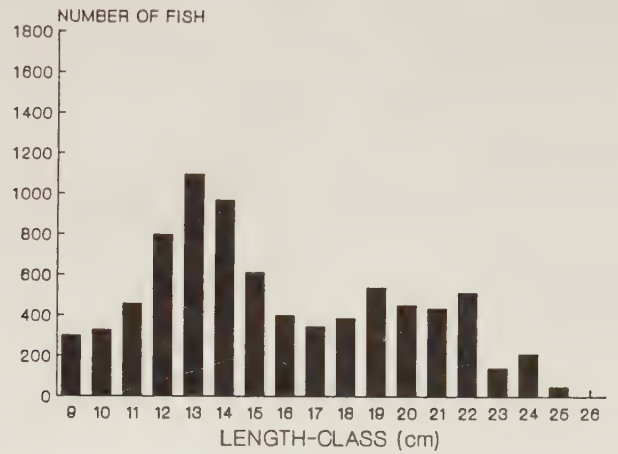
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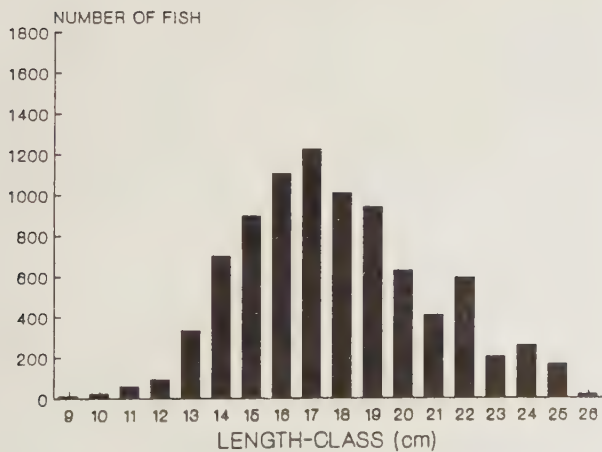
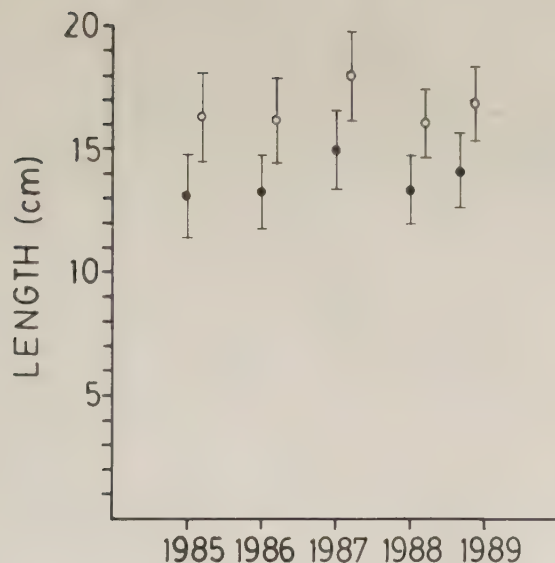


FIG. 3. Estimated number of fish in each centimetre-class, in the length range 9–26 cm, present in Lake Løyning, June 1985–89.

TABLE 2. Estimated number of fish aged 3+ to $\geq 11+$ present in Lake Løyning in June 1985–89.

Year	Number at age:								
	3+	4+	5+	6+	7+	8+	9+	10+	$\geq 11+$
1985	338	1539	2848	1437	1564	987	477	441	145
1986	524	2005	1401	1526	833	1044	363	343	352
1987	1407	528	943	716	661	235	240	150	208
1988	3132	2088	480	460	385	251	110	57	106
1989	1980	3652	1752	318	283	163	220	81	142

FIG. 4. Mean length ($\pm$ sd) for age 3+ brown trout in Lake Løyning at capture in August (1985–88) and September (1989) (open circles) and back-calculated to start of growing season (solid circles).

tionship was obtained using the biomass (kilograms) of age 3+ fish (R_b) and of age 5+ and older fish (B) ($\ln R_b = 5.159 - 0.0038B$; $p < 0.005$, $r^2 = 0.9787$).

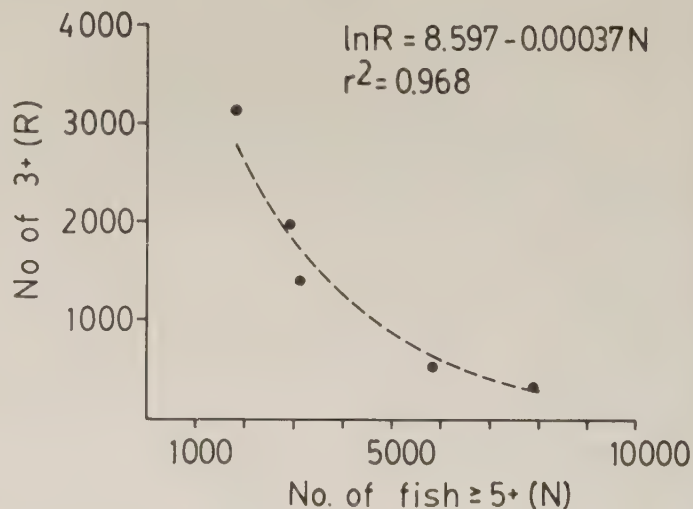
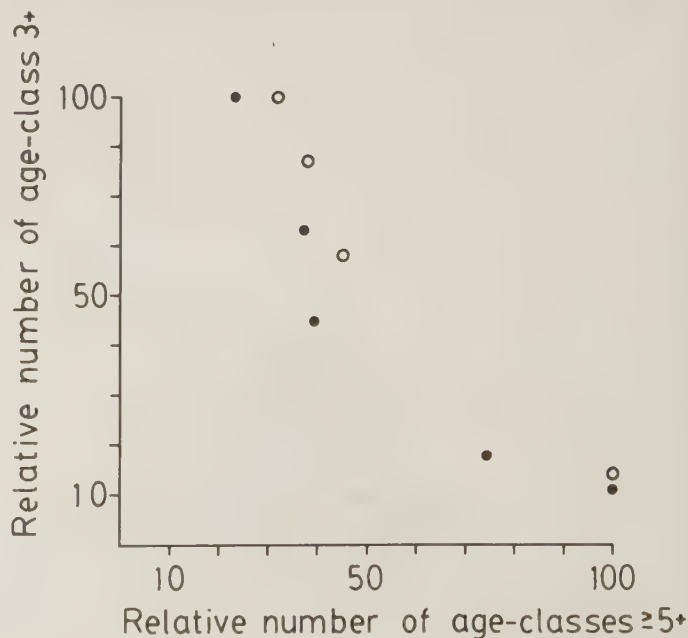
Discussion

Because the brown trout in Lake Løyning spawn in the main river and some small streams, and no lake spawning has been recorded, the results indicate that recruitment from the nursery streams to the lake may be inversely related to the density of the lake population, i.e. at high lake population densities the potential recruits stay in the nursery streams for a longer time, while at low lake densities, more fish enter the lake at a younger age.

The low numbers of smaller brown trout captured by the fine-meshed beach seine in June and the absence of fish in examined brown trout stomachs indicate a very low abundance of the youngest age-classes in the lake. This agrees with results from other brown trout lakes where spawning takes place in streams (Jonsson 1977; Lien 1978).

It is unlikely that the observed recruitment pattern of age 3+ fish during the 5-yr period 1985–89 was a chance occurrence because similar relationships between number of recruits and number in lake population may be deduced from studies of other populations of brown trout (Dahl 1943; Jensen 1977) (Fig. 6).

Also in populations of Arctic char (*Salvelinus alpinus*), density-dependent mechanisms seem to regulate the immigration of young Arctic char from the profundal to the littoral and pelagic zones. The age-classes of Arctic char are segregated in

FIG. 5. Number of age 3+ brown trout present in Lake Løyning in June 1985–89 plotted against number of fully recruited age-classes (age $\geq 5+$).FIG. 6. Relative numbers of age 3+ brown trout present in Lake Løyning (solid circles) and Lake Indre Rødlitjern (open circles) plotted against relative number of brown trout $\geq 5+$ (maximum number set to 100). (Data from Lake Indre Rødlitjern after Dahl 1943.)

different habitats, with the youngest year-classes in the profundal zone and the older fish in the littoral and pelagic habitats (Jonsson and Østli 1979; Klemetsen et al. 1989). When the littoral fraction of old fish in the dense population in Lake Takvatn was heavily exploited, the number of young Arctic char subsequently increased considerably in littoral gillnet catches (Grotnes and Klemetsen 1989). Likewise, recruitment in whitefish (*Coregonus clupeaformis* and *C. lavaretus*) can apparently be stimulated by exploitation (Healey 1980; Amundsen 1988).

Johnson (1972) hypothesised that the main population in unexploited arctic salmonid populations forces smaller fish to marginal habitats. When members of older fish are reduced, the juveniles are likely to move to the most profitable habitat, thereby reducing the intraspecific competition within the marginal habitats, which may be either the profundal zone or streams.

In experiments with sunfishes (Centrarchidae) (Werner et al. 1983; Mittelbach 1984) and threespine stickleback (*Gasterosteus aculeatus*) (Milinski and Heller 1978; Milinski 1985), these fishes quickly responded to increased predation risk or competition by changing their habitat use. Likewise, parr of Atlantic salmon (*Salmo salar*) responded to the sight of a potential predator (brown trout) by moving to a more sheltered habitat (Huntingford et al. 1988). It has been observed that large brown trout often chase smaller individuals and are potential predators (Haraldstad and Jonsson 1983). In Lake Løyning, the small maximum size of the brown trout implies that older individuals are not large enough to prey on 2- or 3-yr-old fish. They may, however, exhibit aggressive behavior, and young brown trout entering the lake may return to the streams when interference in the lake is frequent. In Lake Øvre Heimdalsvatn, a two-way movement of brown trout parr, mainly age-classes 2+ to 4+, has been recorded between the streams and the lake throughout the summer months (Lien 1978).

When the nursery areas are small in relation to the lake, the output of brown trout parr to the lake may balance natural mortality of older fish but be too low to replace an additional fishing mortality. In such situations, intensive exploitation may considerably reduce the total number of fish within the lake, as observed in Lake Øvre Heimdalsvatn (Jensen 1977). However, when nursery areas are large relative to the lake area, as in Lakes Indre Rødlidvatn (Dahl 1943) and Løyning, high exploitation may occur before the lake-resident population number is decreased, due to increased immigration of young fish. If the migration from the streams is delayed by high lake population densities, as suggested in this study, more fish will die during stream residence, resulting in reduced recruitment of a specific cohort to the lake. However, such brown trout populations will have a high ecological resilience, i.e. irrespective of the number of potential recruits in the nursery areas, a density-dependent immigration will tend to keep the number or biomass of the lake population at a more or less constant level, much in accordance with the homeostatic condition described for Arctic char populations (Johnson 1983).

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Seasonal Variation in Spawning by Preovigerous American Lobster (*Homarus americanus*) in Response to Temperature and Photoperiod Manipulation

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By manipulating temperature and photoperiod, we altered the incidence and timing of spawning in preovigerous American lobster (*Homarus americanus*), but the degree of alteration was affected by season. Females moved from natural conditions to unseasonably high temperatures (13–14°C) and short daylengths (LD 8:16) beginning on 27 November did not spawn. Spawning rarely occurred (1%) when these conditions were started on 21 December (winter solstice), but after 8 January, there was a direct relationship between time spent at normal winter conditions and the incidence of spawning after environmental manipulation (8 January, 17%; 23 January, 45%; 8 February, 73%; 3 April, 82%; 12 May, 96%). Spawning occurred 26–42 d after photoperiod and temperature change, and the shortest times were associated with the longest periods of winter conditioning. Furthermore, a relatively brief exposure to normal winter environment enabled preovigerous lobster to disregard daylength and spawn in response to increasing spring temperature. This study indicates that spawning in response to environmental manipulation is altered by season, that duration of exposure to winter conditions influences the timing and incidence of spawning, and that different cues are utilized in different environmental regimes.

En modifiant la température et la photopériode, nous avons perturbé l'incidence et le moment de la fraye chez le homard américain (*Homarus americanus*) préovigère, mais le degré de changement était fonction de la saison. Des femelles n'ont pas frayé après avoir passé de conditions naturelles à des températures anormalement élevées pour la saison (entre 13 et 14°C) et à une courte durée d'éclairement (EO 8:16) à partir du 27 novembre. Dans de rares cas (1%), la fraye a eu lieu lorsque l'on a modifié ces conditions à partir du 21 décembre (au solstice d'hiver), mais après le 8 janvier, on a observé une relation directe entre le temps passé dans des conditions hivernales normales et l'incidence de la fraye après des modifications environnementales (8 janvier, 17%; 23 janvier, 45%; 8 février, 73%; 3 avril, 82%; 12 mai, 96%). La fraye s'est produite de 26 à 42 jours après les changements de température et de photopériode, la période la plus courte avant la fraye correspondant à la période de conditions hivernales la plus longue. Plus encore, une exposition relativement brève à un milieu hivernal normal a permis au homard préovigère de passer outre la durée d'éclairement et de frayer en réaction à une température printanière à la hausse. Cette étude indique que la fraye provoquée par des modifications de l'environnement est tributaire de la saison, que la durée d'exposition à des conditions hivernales influe sur le moment et l'occurrence de la fraye et que, dans différentes conditions environnementales, le homard utilise différents points de repère.

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Photoperiod and temperature influence ovarian development and spawning in the American lobster (*Homarus americanus*) (Nelson 1986; Aiken and Waddy 1989, 1990). On temperature regimes normally encountered by the American lobster in its nearshore habitat, preovigerous females spawn irrespective of photoperiod, but when water temperatures are elevated above seasonal norms, spawning responses are variable and often require a photoperiod stimulus (Aiken and Waddy 1985, 1986a, 1986b, 1989; Waddy and Aiken 1991, 1992).

A previous study (S. L. Waddy, unpublished) suggested that the spawning response of lobster to temperature and photoperiod varied with season. In that experiment, 72 preovigerous females (due to spawn the following July) were transferred from local winter conditions to continuous 13–14°C and LD 8:16 beginning on 21 December (winter solstice). A disease out-

break (gaffkemia) forced the replacement of 20 experimental animals on 10 January. Twenty percent of the replacements (which had experienced an additional 20 d in winter conditions) spawned about 40 d after the thermal increase, but none of the original animals spawned. On 16 February, we shipped preovigerous females from this same stock to the marine laboratory at Bodega Bay, California, where they were placed into temperature and photoperiod conditions similar to those of our experiment. These females had experienced an additional 37 d in winter conditions. Forty-seven percent spawned in the short daylength, but the others spawned only after the daylength was increased (Nelson 1986). These results were inconsistent with earlier studies that indicated preovigerous lobster held through the winter at elevated temperature (13°C) would not spawn if maintained in 8-h photophases (Nelson et al. 1983; Nelson 1986; Aiken and Waddy 1990). It appeared that the animals in

these studies underwent a physiological change after the winter solstice that altered their subsequent response to photoperiod and temperature conditions.

The experiment we report here was designed to resolve the apparent discrepancy and determine if seasonal factors are important in the response of preovigerous lobster to controlled temperature conditions.

Methods

Mature preovigerous lobster that had recently molted and mated were selected from the commercial catch at Miminegash, Prince Edward Island (Canada), in late September of 1988, 1989, and 1990. Female lobster were selected on the basis of molt stage (recently molted) and size (81–85 mm carapace length). With few exceptions, females in this size-class are mature and spawn on an alternate year schedule. Their preovigerous condition was confirmed by examining the cement gland stage of each female (newly molted lobster with stage 1.0 or greater cement glands in the late autumn spawn the following summer) (Aiken and Waddy 1982). As in previous environmental experiments on the same stock (Aiken and Waddy 1985, 1986a, 1989, 1990), lobster were held communally at local seasonal temperature (for temperature record, see Aiken and Waddy 1985) and photoperiod (45°N) from the time of capture in September until the females were transferred to experimental conditions during the winter and spring. The only difference among the groups was the timing of onset of high temperature (13–14°C) and short daylengths (LD 8:16): Group 1, 27 November 1990 ($N = 12$); Group 2, 21 December (three replicates: 2a ($N = 24$), 1988; 2b ($N = 24$), 1989; 2c ($N = 12$), 1990); Group 3, 8 January 1989 ($N = 24$); Group 4, 26 January 1989 ($N = 22$); Group 5, 8 February (two replicates: 5a ($N = 22$), 1989; 5b ($N = 12$), 1990); Group 6, 3 April 1989 ($N = 12$); Group 7, 12 May 1990 ($N = 24$).

During the experiments, lobster were held in individual glass-fronted cubicles of 0.21 m² in identical light-tight rooms. Flow-through seawater (mean salinity 31‰) was filtered through sand, degassed, and supplied at 2 L·min⁻¹. Temperature was controlled by blending unheated and heated seawater to the prescribed temperature in a mixing valve. The heated water was supplied to all tanks from a common reservoir. Photoperiod (LD 8:16) in each room was maintained with electric time clocks controlling incandescent lamps that provided approximately 22 lm·m⁻² to each cubicle. Lobster were fed five times per week on fresh-frozen squid, shrimp, mussels, cod, mackerel, scallop rims, and herring in rotation.

The numbers of spawning and nonresponding females were compared among groups using a chi-square test for homogeneity.

Results

Group 1, 27 November 1990 ($N = 12$): None of the lobster (0%) spawned during the experiment.

Group 2a, 21 December 1988 ($N = 24$): None of the lobster (0%) spawned during the experiment. One female resorbed her oocyte vitellin as evidenced by the dark green coloration of the ventral abdomen.

Group 2b, 21 December 1989 ($N = 24$): One of the 24 lobster (4%) spawned on 5 February, 46 d after the temperature increase (replicate of Group 1).

Group 2c, 21 December 1990 ($N = 12$): None of the lobster (0%) spawned during the experiment (replicate of Group 1 and 4).

Group 3, 8 January 1989 ($N = 24$): Four of the 24 lobster (17%) spawned. Mean spawning date was 19 February, 42 (SD = 5.3) after the thermal increase.

Group 4, 26 January 1989 ($N = 22$): Ten of the 22 lobster (45%) spawned. Mean spawning date was 7 March, 40 d (SD = 5.6) after the thermal increase.

Group 5a, 8 February 1989 ($N = 22$): Sixteen of the 22 lobster (73%) spawned. Mean date of spawning was 17 March, 37 d (SD = 4.8) after the thermal increase.

Group 5b, 8 February 1990 ($N = 12$): Nine of the 12 lobster (75%) spawned. Mean day of spawning was 18 March, 38 d (SD = 5.1) after the thermal increase.

Group 6, 3 April 1989 ($N = 12$): Ten of the 12 females (83%) spawned. Mean spawning date was 2 May, 29 d (SD = 5.0) after the thermal increase.

Group 7, 12 May 1990 ($N = 24$): Twenty-three of the 24 females (96%) spawned. Mean spawning date was 7 June, 26 d (SD = 5.1) after the temperature increase.

None of the lobster in any of the groups molted during the experiment. Experiments were terminated in mid-September of each year, and at that time, none of the females (whether ovigerous or not) had progressed beyond molt stage D_0 (Aiken 1973). Similar proportions of females were unresponsive to environmental induction in November and December ($p > 0.01$), and similar numbers of females in the April and May groups spawned ($p > 0.01$). Responses in all other groups were significantly different from one another ($p < 0.001$).

Discussion

The response of preovigerous females in this experiment to environmental manipulation varied dramatically with season. Spawning did not occur when exposure to elevated temperature (13–14°C) and short daylengths (LD 8:16) began on 27 November, and when exposure began on 21 December (the winter solstice), only 1% of females spawned. Furthermore, these females did not even spawn at the normal time the following summer. In contrast, when females were exposed to the same environmental conditions beginning 18 d after the solstice (8 January), 17% spawned. Exposure commencing later in the winter and spring resulted in spawning by an even larger proportion of the females (26 January, 45%; 8 February, 73%; 3 April, 82%; 12 May, 96%) (Fig. 1). Not only was spawning induced precociously in these groups, but it occurred as synchronously within each group as it would during the normal reproductive season.

Thus, as in previous studies, unseasonal temperature conditions prior to the winter solstice had long-term disruptive effects on ovarian maturation and spawning in preovigerous females (see Nelson et al. 1983; Nelson 1986; Aiken and Waddy 1989). Several years ago, Nelson et al. 1983; Nelson 1986) recognized that spawning was suppressed in short daylengths (LD 8:16) and warm temperatures (10–15°C). This was corroborated by Groups 1 and 2 of this study (exposed to elevated temperature beginning on 27 November and 21 December). However, such females can be induced to spawn with a daylength increase to >12 h (Nelson et al. 1983; Aiken and Waddy 1990).

Earlier results (Nelson et al. 1983; Nelson 1986; Aiken and Waddy 1990) suggested that long daylengths are required for spawning in the spring if winter temperatures remained above 6–8°C, but not if they dropped below that level for an extended period. However, this study shows that response to spring temperature and photoperiod can be altered by prior environmental

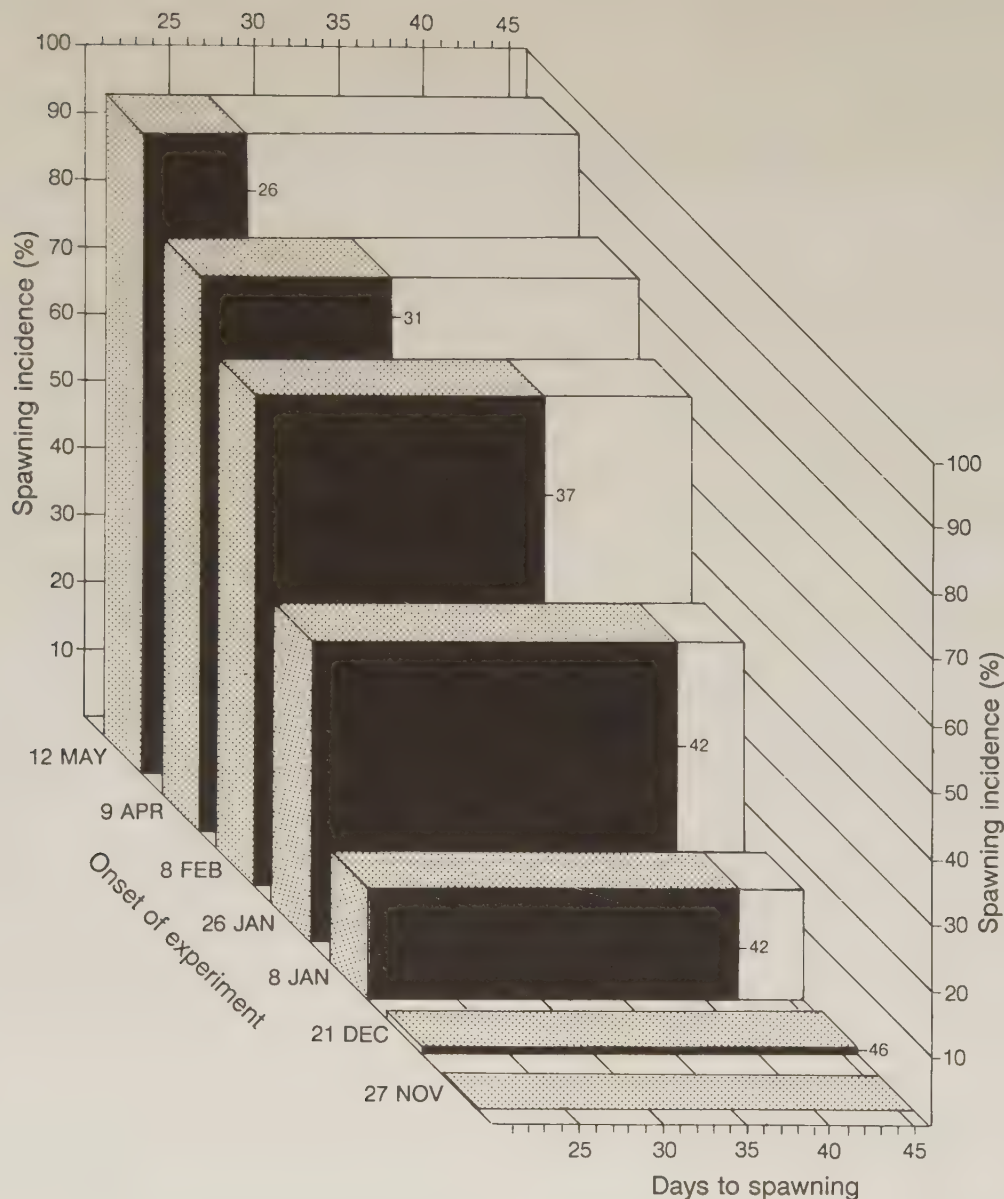


FIG. 1. Seasonal variation in the response of preovigerous female lobster to "summer" temperature (13–14°C) and short daylengths (LD 8:16) during the autumn, winter, and spring.

history as well as season and that shifting the onset of the thermal increase by a few days at the appropriate time of year can produce an entirely different response. It is now apparent that a daylength increase is required for successful spawning only if the thermal increase occurs at or before the winter solstice.

The responses of Groups 1 and 2 were very different from Groups 3–7. This appears to be due to their different environmental history, especially the conditions following the winter solstice. We know from earlier results that switching abruptly from an 8-h to a 12-h photophase provides a long-day cue that favors spawning in females held at elevated winter temperature and that 12 h is the minimum inductive photophase when an abrupt shift is made (Aiken and Waddy 1990). Since the photophase (civil twilight) experienced by the experimental groups on 8 January, prior to the thermal increase, was only 10 h, it seems unlikely that the animals in this study were responding to absolute length of photophase following the solstice. It also seems unlikely that the slight lengthening of the photophase

during the 18-d period acted as a long-day cue, but we cannot dismiss that possibility.

It is clear from this study, as well as from our earlier work (Aiken and Waddy 1986a), that the duration of exposure to winter conditions is important, that once the females have experienced a minimum period of low winter temperature, they become competent to spawn in response to increasing spring temperature regardless of photoperiod. There is a precedent for this in crayfish as well (Aiken 1969).

A similar requirement for an extended cold winter period has been demonstrated in several species of vertebrates and invertebrates, including lizards (Gavaud 1983, 1991), isopods (Madhavan and Shribbs 1981) and insects (Williams 1956; Williams and Adkisson 1964; Saunders 1976). We are currently examining the role of temperature before and after the winter solstice.

It is obvious from these results that female lobster are competent to spawn well before their normal summer spawning period and that the extended period of cold water typical of

nearshore lobster habitat forces preovigerous lobster into a "waiting period" before the onset of final vitellogenesis and spawning. This explains why spawning occurs earlier in warmer areas such as the Gulf of St. Lawrence than in the colder waters of the Bay of Fundy (Templeman 1940) and why female lobster from areas with different spawning seasons spawn at similar times when maintained in a common temperature regime in the laboratory (Waddy and Aiken 1991).

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Embryonic and Postembryonic Development in *Bythotrephes cederstroemii*

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Laboratory observations were made of embryonic development time for parthenogenic *Bythotrephes cederstroemii* under differing temperatures spanning the range when *Bythotrephes* may be present in the plankton of Lake Michigan. Postembryonic development was documented for parthenogenically produced and sexually produced offspring. The complete life cycle of *Bythotrephes* was observed to have two distinct morphological series. Development time from birth to primiparity, consisting of three instars, was 14.0 ± 1.63 d at 12.7°C for parthenogenically produced offspring. Development time at 12.7°C for gametogenically produced offspring was 13.7 ± 1.57 d with four instars. Because parthenogenic eggs released into the brood sac of *Bythotrephes* do not become obvious until the embryo development is well advanced, a useful model for birth rate calculations in field work was developed based on observable morphological traits of the embryos within the brood sac. A curvilinear logarithmic model of development time (D , hours) as a function of temperature (T degrees Celsius) was fit to the embryonic development data: $\log(D) = 6.840 - 7.305 \log(T) + 2.490 \log(T)^2$.

On a observé en laboratoire la durée du développement embryonnaire chez le *Bythotrephes cederstroemii* produit par parthénogénèse, que l'on a soumis à diverses températures comprises dans la plage de températures qui permet au *Bythotrephes* de vivre dans le plancton du lac Michigan. Le développement postembryonnaire des organismes produits par parthénogénèse et par voie sexuée a fait l'objet de travaux de documentation. Les études ont révélé que le cycle reproductif complet du *Bythotrephes* peut se dérouler selon deux processus distincts du point de vue morphologique. Dans le cas des organismes produits par parthénogénèse, le développement à $12,7^\circ\text{C}$ compte trois phases, depuis la naissance jusqu'à la primiparité, et s'effectue en $14,0 \pm 1,63$ jours. Quant aux organismes produits par gamétogénèse, le développement à $12,7^\circ\text{C}$ s'étale sur $13,7 \pm 1,57$ jours et comporte quatre phases. Étant donné que les oeufs parthénogénétiques qui se trouvent dans la poche incubatrice du *Bythotrephes* ne deviennent apparents que lorsque le développement est relativement avancé, un modèle de calcul du taux de natalité a été mis au point d'après les caractéristiques morphologiques observables des embryons situés à l'intérieur de la poche incubatrice; ce modèle de calcul s'avère utile au cours d'études sur le terrain. Un modèle logarithmique curviligne de la durée du développement (D , heures en fonction de la température (T , degrés Celsius), a été appliqué aux données sur le développement embryonnaire de la façon suivante: $\log(D) = 6,840 - 7,305 \log(T) + 2,490 \log(T)^2$.

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The Laurentian Great Lakes have had a history of biological change caused by invading and intentionally introduced species including sea lamprey (*Petromyzon marinus*), rainbow smelt (*Osmerus mordax*), alewife (*Alosa pseudoharengus*), coho (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*), and brown trout (*Salmo trutta*). Whether and how these species have restructured community relationships is a continuing topic of investigation, the study of which has contributed to many alternative descriptions and hypotheses of Great Lakes ecosystem function (e.g. Verber 1966; Beeton 1969; Smith 1972; MacCallum and Selgeby 1987). With the recent invasion of the predatory zooplankton species *Bythotrephes cederstroemii* Schöedler (Crustacea: Cladocera) to the Laurentian Great Lakes (Bur et al. 1987; Lehman 1987; Lange and Cap 1987; Berg and Garton 1988; Cullis and Johnson 1988), much interest is developing in its ecological role in lake ecosystems.

The invasion by *Bythotrephes* permits new tests of hypotheses about community structure. *Bythotrephes* is an invertebrate predator preferring mainly cladocerans (Mordukhai-Boltovskaya 1958). Mordukhai-Boltovskaya (1958) con-

cluded that *Bythotrephes* may be as significant as pikeperch (probably *Lucioperca lucioperca*) in predation on zooplankton in the Rybinsk reservoir. De Bernardi and Giussani (1975) concluded that *Bythotrephes* and *Leptodora* are responsible for a midsummer collapse of *Daphnia* populations in Lake Maggiore. Models to predict the impact of *Bythotrephes* in the Laurentian Great Lakes (Scavia et al. 1988; Sprules et al. 1990) come to two different conclusions, one supporting an impact on community structure and one indicating that major impact is unlikely.

Life history data are essential for developing models of population dynamics. Most reports of *B. cederstroemii* in European (Palearctic) lakes have concerned its taxonomy (Ischreyt 1934; Mordukhai-Boltovskoi 1968; Zozulya and Mordukhai-Boltovskoi 1977). The paucity of ecological studies is in part due to the difficulty of culturing *Bythotrephes* (Mordukhai-Boltovskaya 1957; Berg and Garton 1988). *Bythotrephes* is known to be a cyclical parthenogen. It reproduces parthenogenically during the summer (Mordukhai-Boltovskaya 1957) and gametogenically later in the year. The parthenogenically produced young develop in a fluid-filled brood sac that ruptures to release

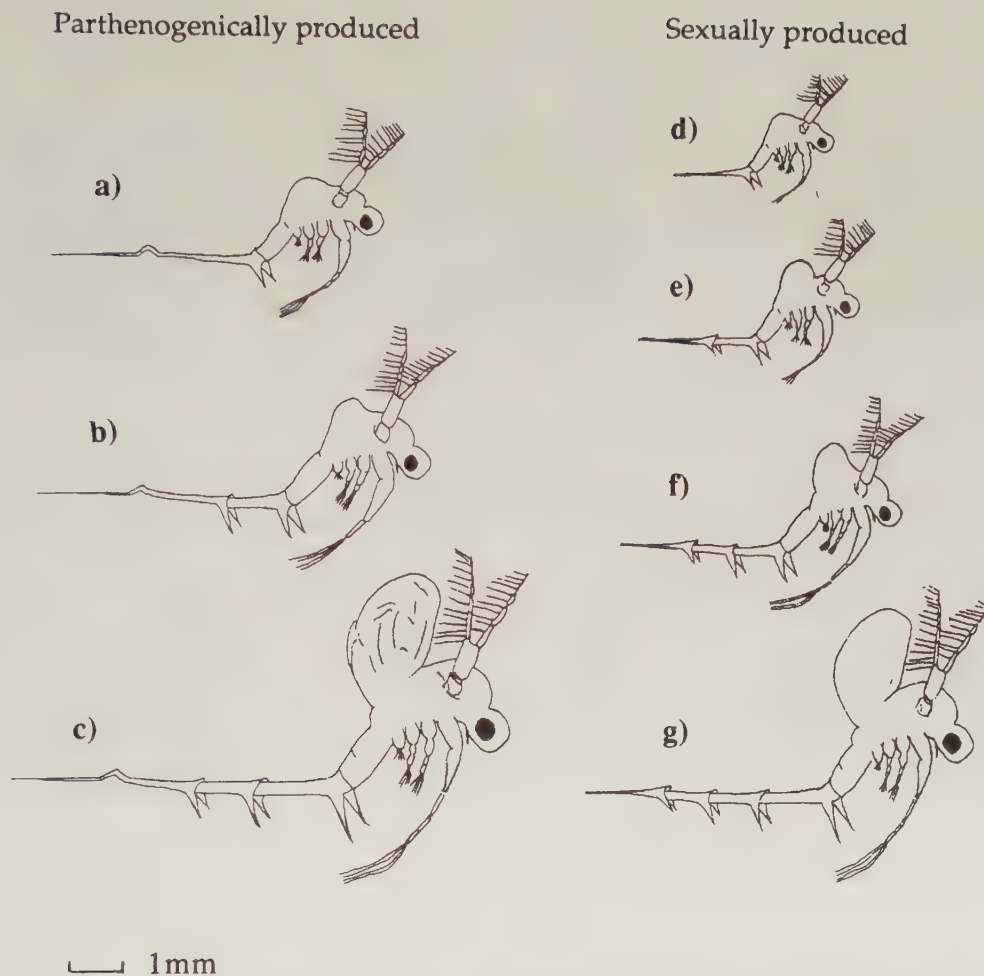


FIG. 1. Morphology of *Bythotrephes* instars produced parthenogenically ((a) neonate, (b) juvenile, and (c) adult) and gametogenically ((d) neonate, (e) and (f) juvenile, and (g) adult).

the young. Although Mordukhai-Boltovskaya (1957) studied parthenogenic reproduction in *Bythotrephes*, her development time estimates are sketchy (11–14 d at 13–16°C), and it is unclear whether she used *B. longimanus* or *B. cederstroemii*. She did not report the number or duration of instars during development. Gametogenic reproduction results in resting eggs with distinct hard thick yellow shells (Herzig 1985) that are released when the broods sac ruptures and which overwinter on the lake bottom. After a refractory period, development proceeds and neonates begin hatching in the early spring (Herzig 1985).

This paper presents experimental observations of development rate of parthenogenic embryos, the instar stages of the *Bythotrephes* life cycle, and development time to primiparity. Embryo development time is mathematically modeled as a function of temperature for computation in population growth dynamics.

Materials and methods

Resting Egg Development

In August 1989, gametogenic females with resting eggs were sorted into tissue culture trays to collect resting eggs for laboratory experiments. The released eggs were transported to Ann Arbor, Michigan, and subsequently separated into individual wells. Eggs were stored at 4°C in the dark to pass through a refractory period (>2 mo; Herzig 1985). Water in

the culture wells was changed monthly (Lake Michigan water filtered through GF/F Whatman filters).

Resting eggs were removed from storage on 7 January 1990 and stimulated by light/dark (L/D) cycles and increased temperature to induce egg development and hatching of sexually produced animals. Four experimental treatments of photoperiod and temperature were used: (1) 10.6°C with 0:24 L/D (wrapped in foil), (2) 10.6°C with 13:11 L/D ($2 \mu\text{E}/\text{m}^2 \cdot \text{s}$), (3) 5.8°C with 0:24 L/D, and (4) 5.8°C with 13:11 L/D. Egg development was observed microscopically and recorded at periodic intervals. As animals hatched, they were removed to another treatment for instar development studies.

Embryogenesis

Experiments were conducted in the summers of 1989 and 1990 onboard the R/V *Laurentian* on Lake Michigan and in the laboratory at the University of Michigan during the summer of 1990. *Bythotrephes cederstroemii* were collected in vertical hauls from the upper 40-m strata at various sampling stations using a 1-m Puget Sound closing net with 130- μm mesh aperture and a solid 2-L PVC cod-end. *Bythotrephes* are phototactic, and as the live animals swam to the top of the cod-end bucket after each tow, they were sorted by hand using either wide-mouth pipets or jeweler's forceps applied to the tail spine. The reproductively mature stage (three-barbed females; Ischreyt 1934) were placed into individual 2-mL wells of tissue culture trays containing filtered (1.2 μm) Lake Michigan water.

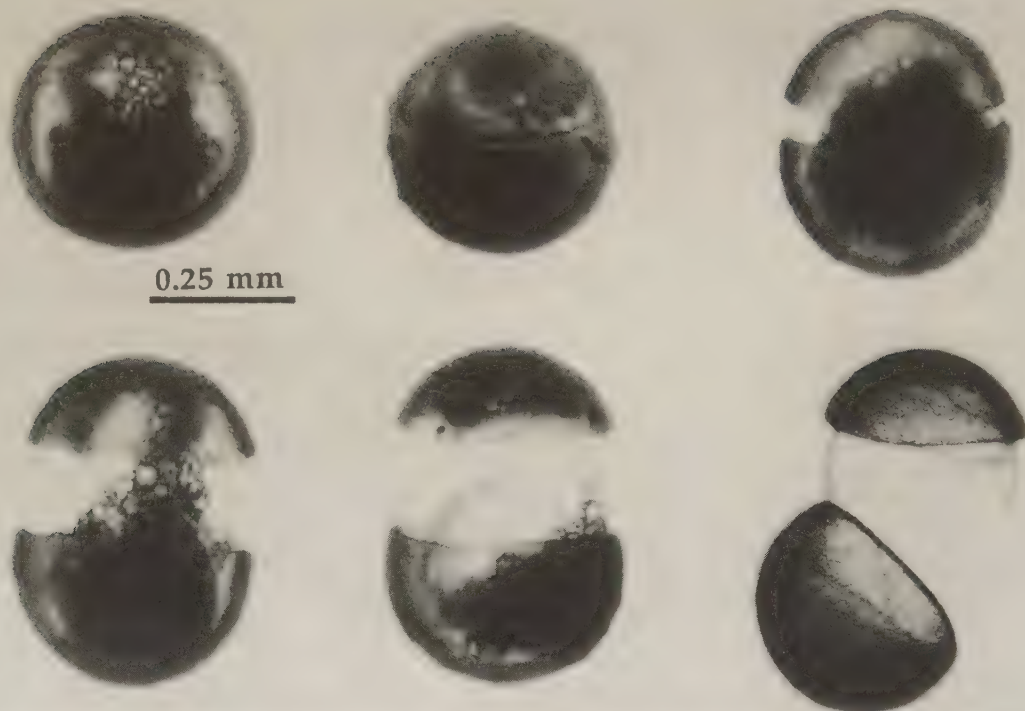


FIG. 2. Development sequence of resting eggs of *Bythotrephes* observed in this experiment depicting unique pop-up stage.

Onboard ship in 1989 the tissue culture trays were kept in a circulating water bath to maintain constant temperature at either 15 or $21.5 \pm 0.1^\circ\text{C}$; in 1990, they were kept in refrigerators regulated by microprocessor to maintain programmed temperature levels of 16 and 20°C . In Ann Arbor the experiments were conducted in culture rooms controlled at 10, 12, and 14°C . In 1989 the animals were not fed as a consequence of the difficulty in maintaining *Bythotrephes* cultures. As a result, the observation period was intentionally kept short to preclude starvation effects, although Mordukhai-Boltovskaya (1958) claimed that *Bythotrephes* may live several days without eating. In 1990, after developing experience it was possible to maintain *Bythotrephes* cultures. This allowed for extended duration of observations, particularly at low temperatures where development was expected to be slower. *Bythotrephes* were fed *Artemia* nauplii rinsed in lake water and the water was changed in each chamber (Lake Michigan water filtered through GF/F Whatman filters) twice daily to maintain healthy animals. Observations in 1990 were conducted over several days to encompass the entire development from the early embryo stage to release of neonates. Animals were observed microscopically at intervals of 4 h or less to record progress of embryonic development at temperatures equal to or greater than 14°C and 6 h or less for the lower temperatures. The culture wells greatly facilitated observations without the need to handle the animals.

Lake Michigan temperature profiles were recorded in real time with a Seabird profiling CTD (conductivity-temperature-depth instrument package). Temperatures for development rate experiments (1989) were initially selected to be representative of depths from which the animals were collected. Subsequently the temperature range was expanded to cover the lower limit at which *Bythotrephes* have been observed in Lake Michigan (Lehman 1988).

Development of the embryo was separated into three characteristic stages: I, "early embryo" during which the initial growth and formation occur and the embryo becomes visible; II, a "red-eye" stage in which a pigmented eye with a reddish color (varying throughout the stage from very faint to dark red) against a white background; and finally III, a "black-eye" stage in which the embryo is well developed and has a black eye. Stage III ends with rupture of the brood sac and release of neonates. Transition from one stage to the next was continuous, and stages could not be readily differentiated at less than 4-h intervals. Longer time intervals limited the precision of defining the time at which hatching events occurred. Therefore, 4 h was chosen as the basic observation interval. However, as a birth became imminent, more frequent observations were made and several births were witnessed.

Instar Development

Neonates hatching from sexually produced resting eggs were transferred, using a widemouth pipet, to individual 2-mL tissue culture wells containing filtered (Whatman GF/F) Lake Michigan water. These were maintained in a culture chamber kept at 12.7°C and 13:11 L/D cycle. Twice daily the water in each well was changed and the *Bythotrephes* were fed *Artemia* nauplii ad libitum. Development stages were observed and recorded. The eventual parthenogenic offspring (F1 generation) from these animals were removed, using a widemouth pipet, and placed in individual wells to prevent cannibalism and for further developmental observations of parthenogenically produced animals.

Parthenogenically produced offspring (F1 generation) from the sexually produced animals and subsequent generations (F2 and F3 generations) from parthenogenically produced animals were cultured using the same conditions and procedures as the

sexually produced offspring from resting eggs. Development stages were observed and recorded.

Results

General Observations

Parthenogenic young of *Bythotrephes* do not develop from yolk-rich eggs as do other cladocerans. Ameiotic eggs are deposited into the mother's brood sac without a shell or yolk and are nourished by fluids surrounding the eggs. During development the embryos increase in size and greatly distend the brood sac. When fully developed, they break out of the brood sac within a span of 1 or 2 min and are released as neonates. Total embryo development time is the elapsed time from the initial deposition of eggs in the brood sac until release of neonates. However, unlike other cladocerans, the release of eggs into the brood sac of *Bythotrephes* is not an easily observable event because the eggs are initially small and inconspicuous (Mordukhai-Boltovskaya 1957; this study).

Most cladocerans molt between clutches and deposit new eggs in the brood chamber (Balcer et al. 1984). In *Bythotrephes*, the neonates break out through the brood sac. In the 1989 studies, molting occurred after birth in 25 of 33 females within the period of observation (five died immediately after release of neonates; $n = 38$). Molting frequency was not recorded in the 1990 studies. Whether or not new eggs are deposited at the time of the molt is not known, mainly because the release of ameiotic eggs into the brood sac is not obvious.

Females with two pairs of barbs (Fig. 1) are typically immature, but occasionally they have been observed as fecund (Mordukhai-Boltovskaya 1957; this study). During the 1989 studies, two of 14 two-barb-stage *Bythotrephes* were observed to be reproductive. Those two are included in the data reported here.

All progeny (1989 and 1990) from both parthenogenic ($n > 1500$) and gametogenic reproduction ($n > 100$) were observed to have only one pair of barbs (Fig. 1) on the caudal spine. This observation conflicts with Mordukhai-Boltovskaya (1957) who reported two- and three-barbed neonates. Berg and Garton (1988) reported a single pair of barbs on neonates they have observed.

Resting Egg Development

After a long period including the refractory time and initial development, the resting eggs undergo a unique pop-up stage (Fig. 2) in which a suture line forms around the egg about one third the distance from a polar end. The asymmetric shell segments partially separate and are maintained in this expanded position by a thinner membrane (Fig. 2). The embryo continues development, progressing to a red-eye stage (II) and a black-eye stage (III) before hatching.

At 10.6°C, animals hatch earlier than those at 5.8°C treatments (Fig. 3). Similarly, in the L/D treatments, animals hatch earlier with the photoperiod than the dark treatment at an equivalent temperature. Development from the pop-up stage proceeds at rates dependent on temperature (Table 1), but independent of light (t -tests, $P > 0.2$).

Embryogenesis

Durations of initial experiments in 1989 were insufficient for most early stage embryos (I) to mature through all stages to neonates. At 21.5°C, the release of 23 clutches was recorded,

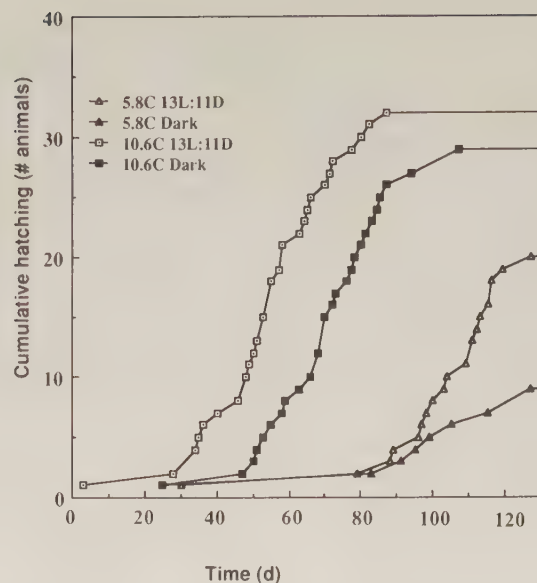


FIG. 3. Cumulative hatching success of resting eggs in response to different temperature and L/D treatments.

although only one clutch was followed all the way from the early embryo stage (I) to neonates. Morphological differences among the stages allow partitioning of embryonic development into distinct intervals which may be added together to reconstruct the entire development time. The repeated observations of developing clutches permitted estimation of the various stage durations. For example, clutches passing from early embryo (I) to black-eye stage (III) during the experiment were observed to complete the entire red-eye stage, (II), although they may not necessarily have been released as neonates by the end of the experiment. Similarly, clutches passing from red-eye stage (II) to neonates completed the entire black-eye stage (III).

At 15°C, complete development through the black-eye stage (III) was observed only once. The interval was 24.67 h. An estimate of ± 6.09 h for the standard error was made from the normalized standard error (SE/X) of the black-eye development stages at the other experimental temperature treatments. Nine clutches passed through the entire red-eye development stage, with stage II being 18.26 ± 3.06 h. An estimated development time from the beginning of the observable red-eye stage is the sum of these two values, 42.93 ± 6.82 h.

At 21.5°C, stage III was found to equal 21.95 ± 3.65 h ($n = 7$) and stage II to be 10.61 ± 2.14 h ($n = 8$). Total development time from the beginning of the red-eye stage is 32.56 ± 4.15 h. Some systematic errors may be present in these estimates because the endpoints or transitions between stages I and II and between stages II and III are not sharply defined.

Success in culturing *Bythotrephes* during the winter months permitted extended observations of embryo development times in 1990. Observations were made during the entire development from the early embryo stage, through the red-eye stage, through the black-eye stage, and finally to release of neonates. Because initial deposition of eggs into the brood sac is not readily observable in living animals, all results are subsequently reported using the distinct morphological development marks of the red-eye or black-eye stage. Development time from the onset of the red-eye stage to birth of neonates at all experimental temperatures is reported in Table 2.

TABLE 1. Chronology of morphological developmental stages of *Bythotrephes* resting eggs under various experimental treatments (days $\pm$ SD to hatching).

Temperature (°C)	L/D	Pop-up stage	Red-eye stage	Black-eye stage
10.6	13:11	7.42 $\pm$ 1.86 (n = 24)	4.56 $\pm$ 1.12 (n = 25)	2.00 $\pm$ 0.59 (n = 18)
	Dark	7.86 $\pm$ 2.10 (n = 22)	4.75 $\pm$ 1.92 (n = 20)	2.25 $\pm$ 1.24 (n = 16)
	Combined	7.63 $\pm$ 1.97 (n = 46)	4.64 $\pm$ 1.51 (n = 45)	2.12 $\pm$ 0.95 (n = 34)
5.8	13:11	12.89 $\pm$ 2.66 (n = 19)	8.06 $\pm$ 1.76 (n = 18)	4.35 $\pm$ 2.01 (n = 20)
	Dark	14.00 $\pm$ 2.96 (n = 9)	8.57 $\pm$ 2.51 (n = 7)	5.38 $\pm$ 2.00 (n = 8)
	Combined	13.25 $\pm$ 2.76 (n = 28)	8.20 $\pm$ 1.96 (n = 25)	4.64 $\pm$ 2.02 (n = 28)

TABLE 2. Development time ($\pm$ SD) spent in red-eye and black-eye stages by parthenogenic embryos under differing temperature treatments. The standard errors for 15 and 21.5°C are computed from separate observations of red-eye stage II and black-eye stage III (see text). The standard errors for all other temperatures are computed from the onset of red-eye stage II until neonate release.

Temperature (°C)	Development time (h)	Sample size (n)
10	106.8 $\pm$ 9.48	25
12	69.62 $\pm$ 14.07	19
14	60.81 $\pm$ 7.73	40
15	42.9 $\pm$ 6.82	8 (II), 1 (III)
16	47.05 $\pm$ 8.79	35
20	37.16 $\pm$ 8.09	15
21.5	32.6 $\pm$ 4.15	8 (II), 7 (III)

Postembryonic Development

The sexually produced neonate has a short straight tail spine (Fig. 1) unlike the characteristically kinked tail spine of the mother (and of parthenogenically produced neonates). The neonate progresses through three molts to become an adult. At each molt the animal sheds its exoskeleton to the base of the tail spine. A new pair of proximal barbs and the growth of an intercalary segment are inserted between the existing tail spine and the body. The previous tail spine is retained intact. A total of four barb pairs was observed in adults. A total of 16 of 19 females was observed with straight four-barb spines after three molts. The three gametogenically produced anomalies were observed to proceed through 3 molts but did not add barbs at one molt; one failed to add barbs at the second molt and two failed to do so at the third molt. The fact that animals may occasionally molt without adding barbs may be responsible for the obser-

vations that parthenogenically produced animals have been observed to reproduce having only two barbs (Mordukhai-Boltovskaya 1957; this study).

The first instar lasts for 3.21 $\pm$ 0.47 d (n = 42) at which time the second pair of barbs is added (Table 3). The brood sac begins to distend in the two-barb stage and continues to enlarge throughout the next instars into the four-barb stage until birth of neonates. The second and third instars follow quickly in 2.56 and 3.23 d, respectively, at 12.7°C. Primarity is reached after an elapsed time of 13.70 $\pm$ 1.57 d (Table 3).

Parthenogenically produced neonates have tail spines that are distinctly longer than those from sexually produced neonates. The total length from the tip of the tail to the barb pair was measured to be 3.88 $\pm$ 0.33 mm (n = 15) for parthenogenically produced animals compared with 1.64 $\pm$ 0.11 mm (n = 15) for gametogenically produced animals. The parthenogenically produced animals possess a spine with the kink typical of *B. cederstroemi*. The parthenogenic neonate is larger at birth and progresses through two molts to become an adult. At each molt, females add a pair of barbs to the tail spine for a total of three barb pairs as an adult. Males do not add a barb at the second molt and have only two barb pairs as an adult. The females brood sac begins to distend in the two-barb stage and continues into the three-barb stage until birth of neonates at 14.0 d at 12.7°C (n = 10) (Table 3). The parthenogenic data include F1, F2, and F3 generation animals. F4 generation offspring were obtained as well.

Discussion

The results of this study describe the life cycle of *Bythotrephes* and permit construction of models for embryo development and durations of instar stages. Two unique features of

TABLE 3. Mean cumulative time in days ($\pm$ SD) for sexually or parthenogenically produced *Bythotrephes* offspring to progress through instar stages to primarity at 12.7°C.

Offspring type	First molt	Second molt	Third molt	Primarity
Sexual	3.21 $\pm$ 0.47 (n = 42)	5.77 $\pm$ 0.71 (n = 26)	9.00 $\pm$ 1.15 (n = 19)	13.70 $\pm$ 1.57 (n = 10)
Parthenogenic	5.39 $\pm$ 1.15 (n = 44)	9.18 $\pm$ 1.49 (n = 28)		14.00 $\pm$ 1.63 (n = 10)

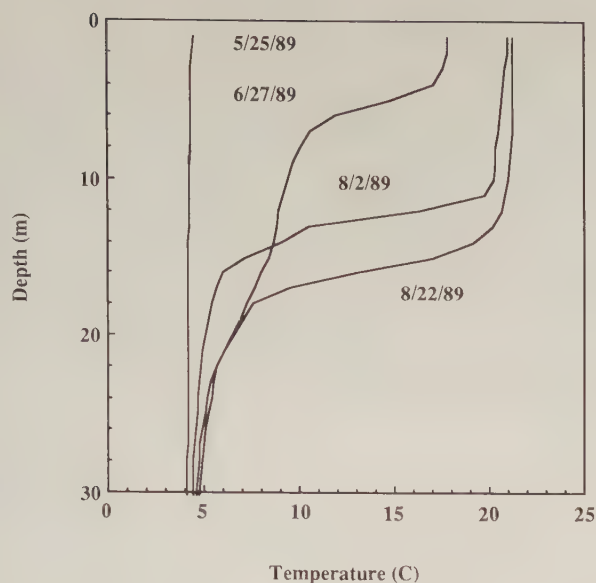


FIG. 4. Temperature profiles at a reference station (102 m depth) in Lake Michigan described in (Lehman 1988) during summer 1989.

the *Bythotrephes* life cycle were observed, a pop-up stage in resting egg development and the retention of the caudal tail spine during molting.

Embryo development times measured with respect to distinct morphological development features were observed from collections of parthenogenic females. Experimental treatments were selected to span the environmental temperatures typically encountered when *Bythotrephes* were present in Lake Michigan plankton (Lehman 1988). These could be compared with successive temperature profiles collected in 1989 (Fig. 4) at an offshore (102 m depth) reference sampling station (43°N, 86°40'W; Lehman 1988).

Temperature has a large effect on the developmental rate of embryos and on subsequent estimates of population growth potential. A prevalent method of modeling the effect of temperature on development time is to use a linear relationship between reciprocal development time and temperature (Edmondson and Winberg 1971):

$$(1) \quad 1/D = m \cdot T + b.$$

Other researchers (e.g. Bottrell 1975; Pastorok, cited in Edmondson and Litt 1982) reported curvilinear relationships to be a better description of the data. Bottrell et al. (1976) recommended that a logarithmic transformation should be used in future work. The form they recommended is one suggested by Bottrell (1975):

$$(2a) \quad \log(D) = a + b \log(T) + c(\log(T))^2.$$

However,

$$(2b) \quad \log(D) = a' + c'(\log(T))^2$$

may be used with only minimal loss of predictive power (Bottrell 1975).

The temperature effects are modeled from the experimentally obtained development times referenced to the morphologically distinct red-eye embryo stage (II) using both the linear relationship (Eq. 1; $m = 0.00175$, $b = -0.00684$, $R = 0.97$) and the logarithmic transformed curvilinear model (Eq. 2a; $a = 6.840$, $b = -7.305$, $c = -2.490$, $R = 0.98$) (Fig. 5). At typical epilimnion summer temperatures (Fig. 4), both models

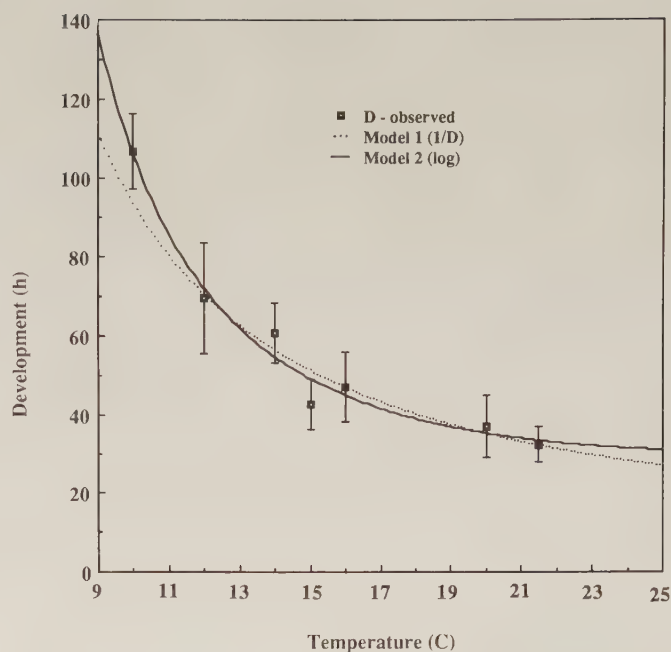


FIG. 5. Mathematical models of *Bythotrephes* embryo development as a function of temperature based on data from this experiment fit to either a reciprocal development relationship (Edmondson and Winberg 1971) (Model 1, $1/D = 0.00175(T) - 0.00684$) or a curvilinear logarithmic relationship (Bottrell 1975) (Model 2, $\log(D) = 6.840 - 7.305 \log(T) + 2.490 \log(T)^2$). D = development time.

predicted similar development times. However, the models diverged at both lower and higher temperatures, with the logarithmic model lying closer to observed development times at lower temperatures.

Although this mathematical model does not encompass the entire embryo development period, it is the most useful formulation for field work because the distinct eye stage serves as an observable time reference. This circumvents the difficulty in observing egg deposition. Methods used to estimate instantaneous birth rates (Edmondson 1971; Caswell 1972; Paloheimo 1974) can be applied by using the pigmented eye spot as a morphological marker. This procedure is preferable in any event because of the recommendation by Threlkeld (1979) to use late-stage embryos in birth rate calculations.

In contrast with embryonic development, only one temperature treatment was followed for instar development time. Modeling the duration of juvenile stages at various temperatures is not yet possible. However, linear translation of axis in the log-log frame of reference of embryo development rates to postembryo development can be scaled to the 12.7°C observation to allow for some hypothetical estimates of the generation time of *Bythotrephes* as a function of temperature (Fig. 6). These may be useful in population growth calculations, if their shortcomings are acknowledged.

Offspring produced both gametogenically and parthenogenically when reared under the same laboratory conditions yielded repeatable results. There is a distinct difference in the number of instar stages to primiparity between the parthenogenically and gametogenically produced animals (three and four instars, respectively). An early season (June) investigation of Lake Michigan in 1990 using a 1-m² Tucker trawl with 300-μm mesh to sample large amounts of water confirmed the laboratory results. The four-barbed gametogenically produced form was observed in these samples. Vekhov (1981) reported *B. longimanus* from tundra lakes with four barb pairs. The sex-

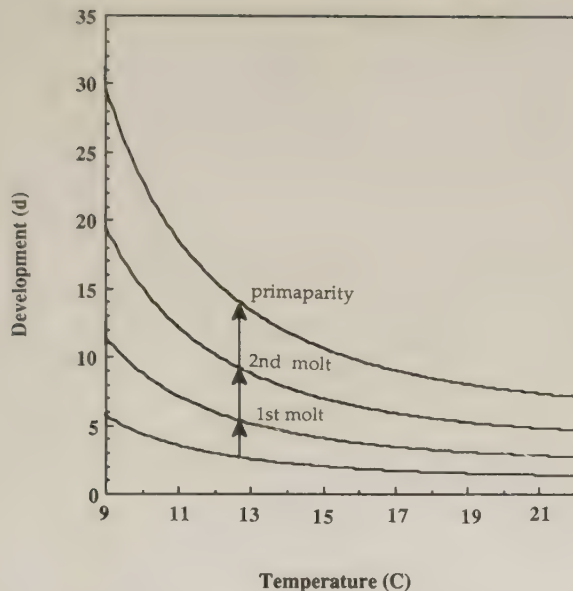


FIG. 6. Hypothesized development times (D') in *Bythotrephes* extrapolated from embryo development times. A translation of axis for the curvilinear logarithmic model is made in the log-log frame of reference ($\log D' = \log D + h$) to the observed development times (D) of the first molt, second molt, and primaparity at 12.7°C. The results are plotted on a linear scale for ease of interpretation.

ually produced animal is much smaller and its postembryonic development requires an additional instar (Fig. 1).

There is a difference between the tail spine in parthenogenic and sexually produced animals. Zozulya and Mordukhai-Boltovskoi (1977) reported seasonal morphological variation of tail spines: straight tail spines in cold water and kinked tail spines in warmer water. My observations coincide with early season straight spines (resting egg hatching) and summer kinked tail spines (parthenogenic reproduction). However, the spines are distinctly different, and not just characterized by the presence or absence of a kink. Unless it occurs very early in the development cycle, environmentally influenced phenotypic expression does not appear to be likely, since resting eggs were hatched at 10°C and parthenogenic embryogenesis was observed at 10°C. This is not conclusive, since the resting eggs did pass through a refractory stage at 4°C with very little development, while parthenogenic eggs may have been exposed to higher temperatures prior to the experimental treatment. Both possibilities can not be ruled out by this experiment, but, if seasonal variation with temperature occurs, it is expressed very early in development.

The pop-up stage in *Bythotrephes* resting egg development is a unique feature that has not been observed in other cladoceran resting eggs. Its functional role may be hypothesized as providing a path for water circulation during respiration, room for expansion as the developing embryo increases in size, and a ready-made shell separation plane for emergence of the hatchling. An interesting question is whether or not the developing embryo controls the mechanism for the formation of this pop-up stage.

The species *B. longimanus* is not available for study in North America and similar life history studies have not been made. Comparative information would be useful for resolving the distinction between *Bythotrephes* species (Bur et al. 1987; Evans 1988).

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Institutional Learning and Spawning Channels for Sockeye Salmon (*Oncorhynchus nerka*)¹

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In the 1960s and 1970s, six artificial spawning channels for sockeye salmon (*Oncorhynchus nerka*) were constructed in British Columbia. I use the evaluation of these facilities and the response to the evaluation to test the hypothesis that fisheries management agencies can learn from experience. One of the facilities was almost immediately determined to be successful, but it took approximately 20 yr for the agency to evaluate the success of the other five. The evaluation was ambiguous for three of these. Only when facilities were overwhelmingly successful or total failures did clear answers emerge. The Canadian Department of Fisheries and Oceans (DFO) has experimented, evaluated, and learned about design, construction, and operation of spawning channels. DFO appears to be less successful at using the evaluation of adult production resulting from spawning channels. Learning appears to work best when goals are well defined, experiments can be conducted and evaluated rapidly, and there is a close organizational connection between decision makers, evaluators, and operators.

Dans les années 60 et 70, on a construit en Colombie-Britannique six frayères artificielles pour le saumon rouge (*Oncorhynchus nerka*). J'ai pris comme point de départ l'évaluation de ces installations et les réactions suscitées par cette évaluation pour vérifier l'hypothèse selon laquelle les organismes de gestion des pêches peuvent tirer des leçons des expériences antérieures. Dans l'un des cas étudiés, on a conclu presque immédiatement que l'expérience serait couronnée de succès; cependant, il a fallu une vingtaine d'années à l'organisme de gestion pour évaluer le rendement des cinq autres installations. Trois de ces cas ont abouti à des conclusions ambiguës. Seules les installations qui fonctionnaient très bien ou qui ont subi un échec total ont donné lieu à des conclusions précises. Le ministère des Pêches et des Océans (MPO) du Canada a expérimenté, évalué et analysé la conception, la construction et l'exploitation de frayères artificielles. Il semble que MPO réussisse moins bien à interpréter l'évaluation de la production de spécimens adultes dans des frayères artificielles. Les recherches s'avèrent plus fructueuses lorsque les objectifs sont clairement définis, lorsque l'on peut effectuer les expériences et les évaluer rapidement, et lorsqu'il existe un lien organisationnel étroit entre les décideurs, les évaluateurs et les exploitants.

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One of the key tenets of fisheries management is that we learn by experience: we believe that a management action which results in a good outcome is more likely to be repeated than a management action which results in an undesirable outcome. The critical components of such learning — evaluation and response — may be difficult, but they are, in theory, possible.

The purpose of this paper is to examine institutional learning in one particular circumstance, the construction, operation, and management of artificial spawning channels for sockeye salmon (*Oncorhynchus nerka*) in British Columbia. Spawning channels were chosen as a case study of institutional learning because (1) they involve a very discrete well-defined decision: to build or not to build, (2) spawning channels have been intensively evaluated and the evaluation has often appeared in the primary literature, and (3) the design, construction, and evaluation have all taken place almost exclusively within a single government agency, the Canadian Department of Fisheries and Oceans (DFO).

I will examine the history of spawning channels to answer the following questions: (1) how were channels evaluated, (2) how clear was the evaluation (did clear yes/no answers

emerge from the evaluation), (3) how long did it take to obtain reliable evaluation results, and (4) did the results of the evaluation affect subsequent decisions?

Spawning channel technology will be used to illustrate the problems and potential of institutional learning — the focus of this paper is not about spawning channels, but on how fisheries agencies learn from experience. Nevertheless, in the process of asking about institutional learning, I will address several key questions specific to spawning channels. In particular I will ask whether, given our present knowledge, spawning channels do appear to be an effective way to increase the harvest of sockeye salmon and whether DFO designed and implemented an effective evaluation program.

History of Sockeye Spawning Channels

Sockeye salmon spawn in rivers and streams adjacent to lakes, and in some systems in gravel areas of lakes themselves. The eggs are deposited in the fall, and alevins emerge from the gravel in the spring. They then migrate to the lakes, normally downstream by passive drift, but in some areas by upstream migration. The juveniles usually spend 1 or 2 yr in the lake and then smoltify and migrate downstream to the ocean, where they may spend 1–3 yr. Spawning channels for sockeye salmon were developed in the 1960s as a means of increasing the production

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FIG. 1. Map of British Columbia showing the location of the eight spawning channels mentioned in the text.

of fry to the rearing lakes. A spawning channel is an artificial river with regulated flow, gravel size, and spawner density. Channels are normally constructed as a serpentine set of loops, bulldozed on a graded area, with gravel added, and some form of flow control structure to provide water. In some cases the spawning channels were built to compensate for degraded spawning habitat or in areas subject to flooding and in others areas simply to utilize the apparent underutilized rearing capacity of lakes.

Six channels built in the 1960s and early 1970s (Table 1) provide almost all of the information used in this paper. These include three channels (Weaver Creek, Gates Creek, and Nadina River; Fig. 1) built by the past International Pacific Salmon Fisheries Commission (IPSFC) and now operated by DFO. The Weaver Creek channel was built because of deteriorating river conditions resulting from logging in the watershed (Cooper 1977). The Gates Creek channel was also built to compensate for apparent deterioration of the natural stream. The

Nadina channel was built to add fry to the apparently underutilized Francois Lake. The three channels built by DFO on streams adjacent to Babine Lake on the Skeena River constitute the Babine Lake Development Project (BLDP). This project grew out of biological studies (Johnson 1956, 1958) which indicated that Babine Lake was underutilized by sockeye juveniles because of limited spawning beds near the lake. Information from these six channels and two channels built in the late 1980s is given in Table 1.

The basic assumption behind a spawning channel is that producing more fry will result in more fish available to be caught. This, in turn, requires four assumptions (McDonald and Hume 1984): (1) artificial channels can produce additional fry, (2) the viability of channel fry would be comparable with that of fry produced naturally, (3) the lakes have capacity to support a larger juvenile population, and (4) an increase in the juvenile output from the lake would result in a corresponding increase in the number of returning adults.

TABLE 1. Location, year built, size, flow, and velocity of sockeye spawning channels in British Columbia.

Channel name	Rearing lake	Year complete	Spawning area (m ²)	Flow (m ³ /s)	Velocity (m/s)
Fulton I	Babine	1965	11 426	2.67	0.43
Fulton II	Babine	1971	73 154	3.60	0.52
Pinkut	Babine	1968	33 442	2.34	0.37
Weaver	Harrison	1965	17 429	0.67	0.27
Gates	Anderson	1968	10 995	1.26	0.31
Nadina	Francois	1973	18 131	1.78	0.35
Chilko	Chilko	1988	8 565	2.70	0.38
Horsefly	Stuart	1989	15 456	1.68	0.34

Methods of Evaluation

The biological effects of a spawning channel can be assessed by examining the numbers and quality of the fish produced from the channel and their impacts on numbers and quality of naturally produced sockeye from the associated lake and river system. In practice this means counting the number of adults entering the channel, counting the fry leaving the channel, counting smolts resident in or leaving the rearing lakes, and assessing the subsequent return of adult sockeye to catch and escapement.

Spawners to Fry

All spawning channels have means to limit and count the number of fish entering the channel. Because sockeye spawners are sexually dimorphic the number of females and males is recorded. Females are sampled for fecundity and carcasses are examined for egg retention, enabling estimates of total egg deposition.

Channel operators can, in theory, also control flow, gravel size, and gravel cleaning method and frequency. In practice, flow and gravel size are rarely modified once a channel is constructed, so choice of spawning density and of gravel cleaning frequency and method are the two major decisions facing a channel operator. Several channels have also had postconstruction engineering modifications to reduce sediment deposition.

Fry to Smolt

Once the fry leave the channel, evaluation of channel production becomes very difficult. In all systems, the channel fry mix with naturally produced fry. In the Babine system, smolt output from Babine lake has been measured; estimates of total natural fry production have been made, and detailed marking studies of survival of channel produced fry have been performed (Coburn and McDonald 1972; McDonald and Hume 1984). There have been no fry to smolt studies in any of the

IPSFC channels; estimates of fry production from natural stream spawning have been made (Cooper 1977), but smolts have never been counted.

Smolt to Adult

Adult production of sockeye is the sum of the spawning escapement and the catch. Escapement is estimated for all major sockeye systems, usually by some form of visual counting. Allocation of catch to stock of origin is difficult, particularly from the many mixed-stock fisheries that take place on sockeye. Fish returning to the Babine system are captured in several fisheries in British Columbia and southeast Alaska. Various methods for determining the number of Babine fish caught in these fisheries have been developed (Starr et al. 1984; West and Mason 1987; Starr and Hilborn 1988). These methods, known as run reconstruction, use information about run timing and migration pattern to determine stock contribution.

Fraser River sockeye salmon are caught throughout southern British Columbia and northern Puget Sound. The IPSFC developed a scale pattern recognition system for identifying area of origin of Fraser River fish, but this system is not of sufficient accuracy on a small spatial scale to distinguish the fish from spawning channels from the sockeye produced from natural populations in the lake with the channel. Therefore, evaluation of adult returns to the IPSFC channels have been based solely on escapements.

Evaluation of Babine Channels

Evaluation of the Babine channels was much more thorough than the IPSFC channels. The Babine channels were conceived by researchers and have always had a major research component; the IPSFC channels were conceived as production facilities with little if any research components. The published evaluation history of the Babine projects is found primarily in six papers; McDonald (1969), Dill (1970), Ginetz (1977), West (1978), McDonald and Hume (1984), and West and Mason

TABLE 2. History of published evaluations of the Babine channels. An × indicates that the topic was treated in the paper; weak indicates that the topic was mentioned but not examined in any depth.

Topic	McDonald (1969)	Dill (1970)	Ginetz (1977)	West (1978)	McDonald and Hume (1984)	West and Mason (1987)
Spawner density			×	×		
Fry production	×	×	×	×		
Smolt production				Weak	×	×
Adult production						×
Cost benefits						Weak

(1987). Four of these six papers evaluate only one or two of the six possible topics, and fry and smolt production are the topics most commonly evaluated; stock interaction and benefit: cost ratios are weakly evaluated in any paper (Table 2).

Spawner Density

In most channels, egg to fry survival decreases as the density of spawners increases. Higher spawner densities tend to produce more fry, but have also been associated with outbreaks of infectious haematopoietic necrosis (IHN). Determination of the best spawner density has been an active topic of discussion and experimentation since the first year of channel operations (Ginetz 1977) and remains so today. For most channels there has been a general downward trend in survival over the years, which has also normally been associated with increasing spawner density. The density-dependent egg to fry survival results in a rather wide range of spawner densities that produce roughly the same number of fry. The first two major evaluations of the BLDP were by Ginetz (1977) and West (1978), both of whom were concerned almost exclusively with spawner density and fry production.

Fry Production

The first key question in evaluation of spawning channels was the ability to produce fry of good quality. The number of fry produced was available as soon as the channels went into production; the fry were counted out of the channel the spring after spawning. Biological experiments on relative viability and survival of channel fry were performed as soon as Fulton I began operating (1965) and were published in journals by 1969 (McDonald 1969; Dill 1970; Payne 1971; Ginetz 1972; Scarsbrook and McDonald 1970, 1972; Coburn and McDonald 1972, 1973). All of these studies indicated that the channel fry were as viable as natural fry. Fry production is closely tied with spawner density and outbreaks of disease mentioned above, and fry production was extensively reviewed in Ginetz (1977) and West (1978).

Smolt Production

The next stage in evaluation was smolt production; channels could put fry into the lake, but would this result in additional smolts leaving the lake? Ginetz (1977) did not address smolt production. West (1978) was chiefly concerned with the success of fry production and only considered smolt output in the last three pages of his 100-page report. Smolt production from brood years 1972–75 was disappointing (Fig. 2) and indicated that there might be a serious problem with the assumption that more fry into the lake meant more smolts out. West concluded in his abstract, “When large numbers of fry are produced, however, the smolt output has been found to be less than anticipated.”

Thirteen years after the first channel began operations, the initial evaluation of smolt production was discouraging. Later evaluations (McDonald and Hume 1984; West and Mason 1987) examined smolt production with the benefit of additional years of data and both showed that increased fry into the lake did result quite clearly in more smolts leaving the lake.

Adult Production

Not until the mid-1980s did DFO biologists ask if more adults were being produced because of the BLDP. The adult production story was more complex than that for fry and smolts.

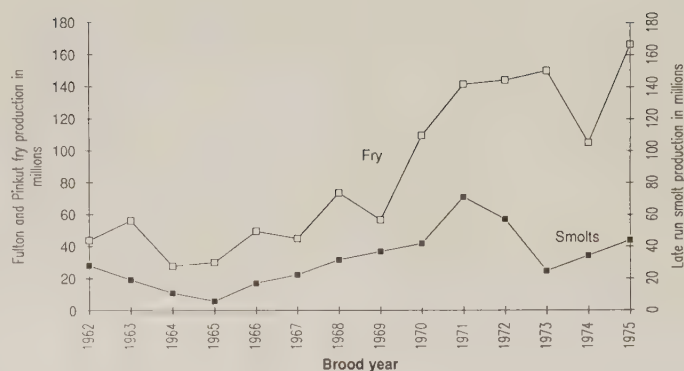


FIG. 2. Babine fry and smolt production from 1962 to 1975. Redrawn from fig. 39 of West (1978).

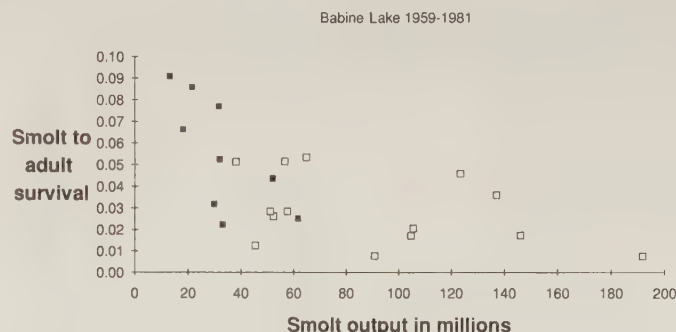


FIG. 3. Smolt to adult survival for Babine sockeye brood years 1959–81. Solid points indicate years before the BLDP; open points are years after BLDP. Data from Macdonald et al. (1987).

Peterman (1978) had published DFO data showing that smolt to adult survival decreased as total smolt numbers increased, thus greatly reducing the anticipated number of adults returning. The data were available in 1978 to examine total adult production, but no one associated with the BLDP was doing such evaluation. The smolt to adult survival versus smolt output data (Macdonald et al. 1987) are shown in Fig. 3.

Neither McDonald and Hume (1984) nor West and Mason (1987) chose to actually plot the adult production data. McDonald and Hume (1984) plotted log smolts versus log adults for even and odd years, and they showed that in odd years there seemed to be a more or less proportional increase in adults for an increase in smolts, while in even years there was no apparent increase. West and Mason (1987) estimated average annual production from the BLDP and all Skeena River stocks for the pre-BLDP period (calendar years 1958–71) and post-BLDP period (1973–85) (Table 3). They estimated that the annual Skeena River total return (Canadian and U.S. catch and escapement) fisheries in the post-BLDP period was 2 453 000 fish, with a net increase of 1 009 000. This was calculated by taking total returns for calendar years 1958–71 and comparing them with total returns for calendar years 1973–85. West and Mason (1987) did not present the year by year data.

Macdonald et al. (1987) presented brood year returns (not including Alaska catch) from brood years 1943–81. The average total return does now appear to be higher in the post-1968 period than before the BLDP (Fig. 4), but using the Macdonald et al. (1987) data the difference between pre-BLDP (1958–68) and post-BLDP (1970–81) is 832 000. Henderson and Diewert (1990) have reconstructed the Skeena River sockeye run from brood years 1965–82 using several methods, all of which include U.S. and Canadian catches, and their estimate of the increase from brood years 1965–68 to brood years 1970–81 is

TABLE 3. Annual pre- and post-BLDP adult sockeye production. Data from West and Mason (1987). All numbers in thousands.

Where adults ended up	Pre-BLDP (1958–71)		Post-BLDP (1973–85)		Skeena increase
	Babine	All Skeena	Babine	All Skeena	
Alaska fisheries	20	77	86	152	74
CDN fisheries	192	693	740	1318	625
Escapement	146	674	534	983	310
Total	358	1444	1360	2453	1009

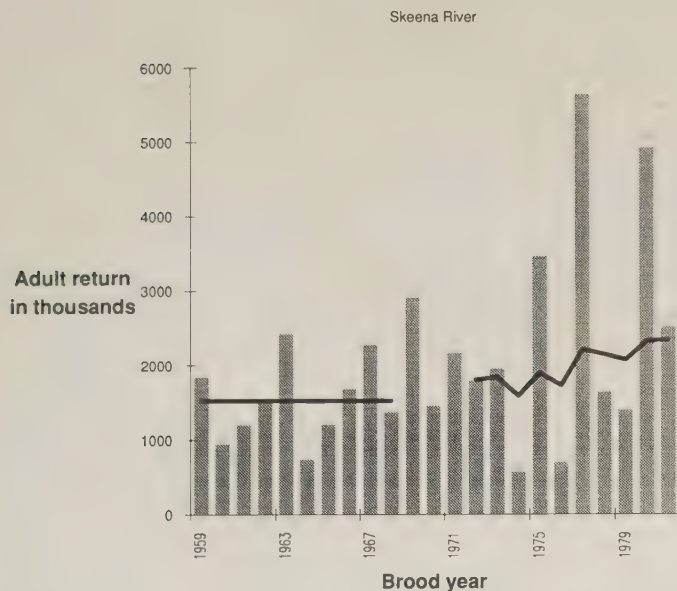


FIG. 4. Total return (Canadian catch plus escapement) of sockeye salmon to the Skeena River by brood year. The solid line before 1969 represents the pre-BLDP average total production; the solid line after 1970 is the running average after 1970. Data from Macdonald et al. (1987). The "postchannel" average return is therefore the solid line for brood year 1981.

540 000 or 750 000, depending upon which method they used. None of these estimates is directly comparable, since West and Mason (1987) used calendar year returns, Macdonald et al. (1987) did not include U.S. catches, and Henderson and Diewert (1990) only began in 1965. Nevertheless it is clear that the total return to the Skeena has increased since the BLDP by somewhere between 500 000 and 1 000 000 fish.

In asking whether the BLDP produced the additional fish, should we simply accept the 1 million estimate? It is not at all clear. This assumes that in the absence of the BLDP, the Skeena and Babine production would have stayed the same. Is this a reasonable assumption?

There are at least two reasons this may not be reasonable. First, the pre-BLDP period was one of recovery from the landslide of 1951, which reduced Skeena stocks to their lowest levels in history (Larkin and McDonald 1968). A major slide into the Skeena River had partially blocked upstream passage, and escapements in the first few years thereafter were reduced. In response, DFO reduced harvest pressure, and the trend in abundance post-1951 was one of increase. This trend might have continued even if the Babine channels had not been built. For this reason, the estimates of post BLDP production from the Henderson and Diewert (1990) data (which use only post-1965 data) are lower than the estimates from West and Mason (1987). Second, a greater challenge to the before and after

BLDP comparison is that the largest sockeye producing systems in the U.S. and Canada also showed significant increases in abundance in the 1970s and 1980s.

Bristol Bay Alaska and the Fraser River are the number 1 and number 2 sockeye producers in North America and both showed proportional increases similar to or greater than the Skeena in the pre- and post-BLDP periods (Fig. 5). One could argue that the Babine has gained no production in comparison with the other large sockeye-producing systems over the same period. I believe we cannot determine how effective the BLDP has been at producing additional sockeye. In the absence of controls, we do not know what would have happened if the channels were not built (and we never will).

Economic Benefits

There remain the questions of net economic benefits (benefit:cost ratio), interaction with other stocks, and effects of increased production on price. West and Mason (1987) addressed all of these questions briefly. They presented some simple benefit:cost numbers to indicate that given an estimate of 600 000 extra fish in the catch (1 000 000 increase in return), the benefit:cost ratio was 3:1, although little documentation for this was provided. In particular, almost all of the extra production came from two brood years, 1977 and 1980. West and Mason (1987) suggested that the benefit:cost ratio was calculated based on an extra \$5.5 million in landings every year in the post-BLDP period. Since the actual increase in production did not occur until about 15 yr after the construction was begun, a more rigorous benefit:cost evaluation (one including discounting for instance) might produce a much lower ratio. McDonald and Hume (1984) presented no analysis of cost effectiveness. Essentially there has been no documented evaluation of the cost effectiveness of the BLDP.

Impacts on Other Stocks

West and Mason (1987) also examined the impacts of the BLDP on non-Babine stocks. They showed that non-Babine stocks declined 40% in the post-BLDP period. This was felt to be due to the reduced ocean survival of all Skeena fish due to large smolt migrations and, for some non-Babine stocks, higher harvest rates associated with fisheries on the larger Babine runs. However, since the Babine stocks constituted over 90% of the Skeena sockeye stock before the BLDP, the decline in non-Babine stocks is not a major factor in total Skeena production. It is, nevertheless, a serious concern for local fishermen, particularly subsistence fishermen on the affected non-Babine stocks. Decline of non-Babine stocks is also of great concern for long-term conservation — few biologists would be comfortable with the loss of the non-Babine stocks.

No one has addressed possible decreases in price due to increased production from the Babine, but given the small pro-

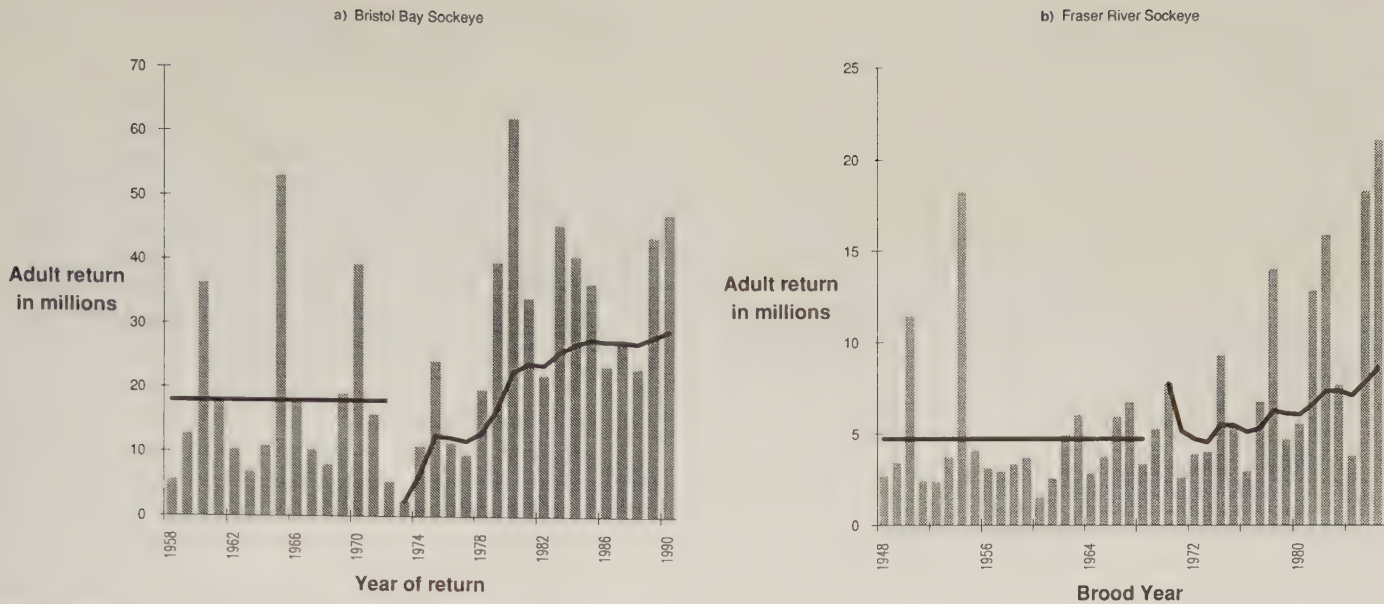


FIG. 5. Total returns (catch plus escapement) of sockeye salmon to (a) Bristol Bay Alaska and (b) the Fraser River. The solid lines are pre-1969 brood year average return and post-1970 brood year running average return to correspond with a similar format in Fig. 4. The Bristol Bay data are by year of return; the Fraser River data are by brood year.

portion of North American sockeye produced by the Skeena, any price depression would likely not be great, and in fact the price of salmon in the late 1970s increased. However, since almost all of the benefits of the BLDP have come from three large return years, there may have been local swamping of processing capacity in those years causing a local reduction in price.

IPSFC Channels

Evaluation of the IPSFC channels has been much less intense than the BLDP channels. Spawning input and fry output are monitored, as is escapement to natural streams. There have been no studies of fry viability or smolt production. The only published evaluation was by Cooper (1977), who reviewed the performance of all of the IPSFC spawning and incubation facilities for sockeye and pink salmon (*O. gorbuscha*). An extensive review of the IPSFC pink and sockeye facilities was contracted by the Salmonid Enhancement Program (SEP) in 1985 and was provided to me by SEP staff.

Cooper (1977) showed that all three channels had produced the expected numbers of fry, but the adult production picture is much different. The Weaver Creek channel has done very well, the escapement now being 47 087 fish larger than it was before the building of the channel (Fig. 6). In contrast the Gates and Nadina channels show much smaller increases in the escapement (8554 at Gates and 16 385 at Nadina); in comparison with Weaver Creek, they are disappointing. It is possible, of course, that the total runs have increased much more than shown by escapement because selective fishing on these stocks might have kept the escapement low, but there is no documentation of such stock-specific increases in fishing pressure.

My evaluation of the IPSFC channels is shown in Table 4. For Weaver Creek, the prechannel (1962–68) escapement was 13 904 and the postchannel escapement 60 991. The net increase in escapement is therefore 47 087. The exploitation rate for Fraser stocks averages about 80%; therefore, there are five additional fish in the adult return for every additional fish in the escapement.

However, there has been an increase in all Fraser stocks, and from 1969 to 1985 the Fraser returns were 1.37 times the average returns from the period 1962–69. The following equation was used to calculate the increase in adult production:

$$\text{Increased adults} = \left(\frac{\text{escapement after}}{\text{increase in Fraser}} - \text{escapement before} \right) \frac{1}{(1 - \text{harvest rate})}.$$

The escapement after channel construction divided by the increase in all Fraser River stocks during this period is an estimate of the escapement corrected for the overall trend in escapements on the Fraser. Subtracting the escapement before channel construction provides an estimate of the net increase in escapement due to channel construction. This is divided by 1 minus the harvest rate to estimate total adult production caused by channel construction. Table 4 shows these calculations for the three IPSFC channels. The numbers for Weaver Creek in Table 4 show why it is considered such a success, over 200 000 fish a year produced per year (at a value of over \$10 per fish) for a few hundred thousand dollars investment. Table 4 also shows pre- and postchannel average wild escapement. For Weaver Creek the postchannel wild escapement was 22 124 higher than the prechannel escapement.

Table 4 shows that Gates and Nadina are much more disappointing. While both channels have shown an increase, even after adjustment, the increases are very small, and for both systems there has been a major drop in the wild spawners. There has always been a problem getting fish into Nadina to spawn, and only once in its history has it been fully loaded. The estimated increases in adult production of 12 134 at Gates and 17 703 at Nadina are much less than anticipated and reflect the almost total failure of these channels to increase adult production.

Cooper (1977) included a benefit:cost analysis of the channels. Unfortunately it is impossible to understand how the adult production was calculated, but he gave benefit:cost ratios for

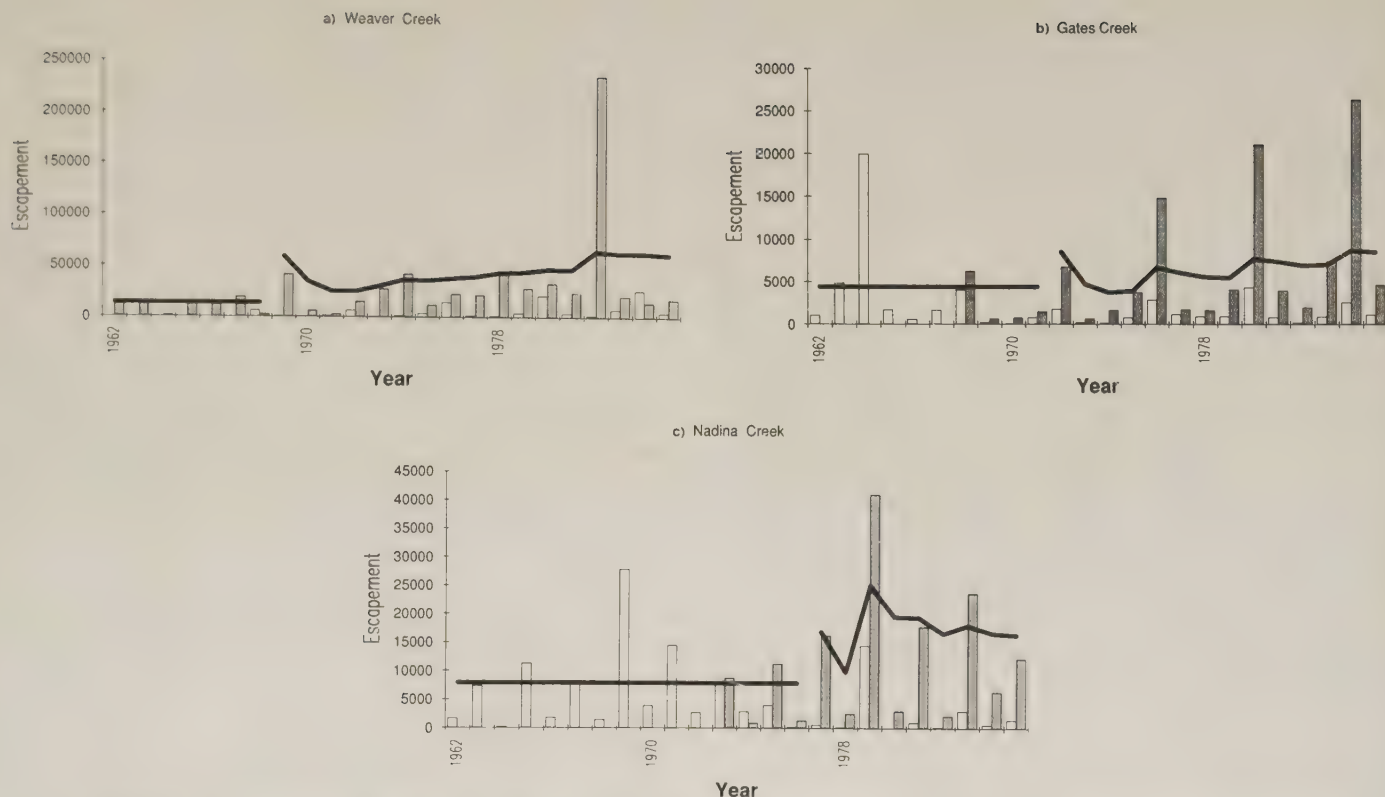


FIG. 6. Escapement to the (a) Weaver Creek, (b) Gates, and (c) Nadina channels on the Fraser River. Solid vertical bars are natural spawners; open vertical bars are fish spawning in the channels. Note that the "postchannel" average escapement is the height of the solid line on the far right-hand edge of the graph.

TABLE 4. History of channel production from Fraser River spawning channels, calendar years 1962–85.

	Weaver	Gates	Nadina
First year of channel loading	1965	1968	1973
First year of channel return	1969	1972	1977
Prechannel average escapement	13 904	4 408	7 880
Postchannel average escapement	60 991	8 554	16 385
Ratio of pre- to postchannel	4.39	1.94	2.08
Unadjusted increase in escapement	47 087	4 146	8 505
Increase in all Fraser stocks in same period	1.37	1.39	1.63
Adjusted increase in escapement	41 943	2 427	3 541
Adjusted increase in total adult production	209 713	12 134	17 703
Postchannel average wild escapement	36 028	1 349	2 422
Net change in wild stock	22 124	-3 059	-5 458

Weaver Creek of 9:1 and for Gates Creek of 2:1. No benefit:cost ratio was given for Nadina. These calculations are totally undocumented. The consultant's report did not address adult production.

Do the Results of Evaluation Affect Subsequent Decisions?

The published evaluations discussed above are only part of the evaluation process; they are the tip of a very large iceberg of studies, data reports, manuscripts, and personal opinion. I have concentrated on the published records because they are publicly available and easily obtained. When we ask if the results of evaluation affect subsequent decisions, we must deal not only with the published evaluations, but with personal opinion. In addition to examination of published reports, I have held

formal and informal interviews and discussions with most senior SEP staff and many DFO biologists inside and outside of SEP.

Consensus about Historical Performance

Fortunately, there appears to be remarkable agreement among salmon biologists and engineers about the effectiveness of channel technology for sockeye. In January 1990, the University of Washington sponsored a workshop on spawning channel technology for sockeye, aimed at utilizing the lessons of British Columbia channels for application to a proposed channel in Washington (Hilborn 1990). Six DFO biologists were present who had extensive experience with channels, including former IPSFC staff, DFO research scientists, and DFO evaluation biologists. The discussion revealed general

agreement that (1) Weaver Creek was an unqualified success, (2) the BLDP had produced additional adult sockeye, but the exact number was uncertain, and (3) Gates and Nadina were almost total failures at increasing the return of adults. Some facilities worked, and some did not.

The less than anticipated increase in Skeena River production from the Babine channels is primarily due to the lower smolt to adult survival that has occurred as smolt production increased. There has been some loss of non-Babine stocks, but most of the problem seems to have been with the smolt-adult survival. Despite the large increases in smolt production, only three brood years (out of 12 considered) have produced significantly improved adult returns. There was some suggestion (Peterman 1978) that this could be an interaction with pink salmon, but West and Mason (1987) pointed out that the apparent difference between even and odd broods shown in McDonald and Hume (1984) had not been supported by data that became available between 1984 and 1987. In short, no one has any idea how to improve production from the Skeena system — smolts have been produced as planned, but adults are not returning at the expected rate.

DFO biologists associated with the BLDP suggested that if there had been more evaluation at Gates and Nadina, particularly if fry survival and smolt numbers had been measured, we might know why there had been no increases there. For instance, if there is a problem with the in-lake growth or survival, whole-lake fertilization and predator control are possible remedial actions. However, in the absence of any postfry studies, no one knows why these two channels have been so unsuccessful.

Using Prior Experience

Let us now see if the information gained from evaluation was actually used. Spawning channels are a comparatively simple technology, and I will concentrate on three decisions: (1) how to clean the gravel, (2) how many fish to put in, and (3) if, when, and where to build additional channels.

Improving channel operation

There are many design and operational decisions to make when building and operating a channel such as choosing gravel size, flow, gradient, width, bottom material, etc. Once a channel is built and operational, almost all of these are fixed. Gravel cleaning is probably the most difficult operational problem for channel operators; the accumulation of silt, debris, and pathogens in the gravel is widely acknowledged to cause declines in egg to fry survival as channels age. Both DFO and the IPSFC (while it operated the Fraser channels) spent considerable time and money working on gravel cleaning. It is not yet clear if a completely satisfactory gravel cleaning technology has been developed. Most methods tend to redistribute the gravel with smaller particles dropping towards the bottom, and all methods cause significant sediment discharge that may pose water quality standards problems. It is uncertain if current cleaning methods can reverse the declining egg to fry survival rates seen in many channels. However, channel cleaning does show a very healthy level of experimentation, evaluation, and discussion.

Determining how many spawners to allow in a channel involves a trade-off between more eggs deposited and generally lower egg to fry survival as spawning numbers increase. Channel operators have experimented with different spawner densities and found that egg to fry survival decreased at higher densities. This issue is one of the main questions that Ginetz (1977) and West (1978) addressed. Generally, channel opera-

tors have chosen to load the channels at a lower density than would produce the maximum fry output because of concern about disease. IHN has been a problem in some years in several spawning channels (Traxler and Rankin 1989), and this has caused concern with channel operators.

The two operational decisions discussed above, channel cleaning and spawner densities, involve an evaluation–decision structure that works well for three reasons. First, the objective is quite clear — to produce good quality fry in large numbers. Second, the decisions can be quickly evaluated, since the number of fry is measured about 6 mo after the cleaning or spawner density decision is made. There is rapid feedback between decision and result. Third, all the decision and evaluation steps are taken within the channel facility, so that the same people who run the facility are involved in the assessment of fry production.

Predicting production for proposed projects

Do the results of evaluation affect the decisions to build additional spawning channels? The only two sockeye spawning channels that have been built since the completion of the BLDP are the Chilko River channel completed in 1988 and the Horsefly River channel completed in 1989. These are both relatively small channels, 8000 and 15 000 m², relative to the much larger Fulton II (73 000 m²) and Pinkut (33 000 m²). The 17-yr gap between completion of the last BLDP facility and the Chilko River channel provided time to conduct and act on the evaluations. Did DFO learn from the experience of the BLDP and IPSFC channels?

Within the realm of design, construction, and operation the answer appears to be yes. Discussions with DFO staff indicate that the design and construction process went very well — what had been learned in design and operations of the existing facilities was well incorporated into the Chilko and Horsefly channels.

The decisions to actually build the Chilko and Horsefly channels indicate much less learning. SEP maintains a list of “Bio-engineering Standards” which are assumptions regarding anticipated survival through different life stages. The biological standards published for sockeye spawning channels are 24.2 adults/m² of gravel in the Fraser and 10.8 adults/m² in non-Fraser areas. We might expect these standards to reflect the evaluation experience from the published papers discussed earlier.

Remarkably the standards appear to differ considerably from observed data (Table 5). For BLDP, West and Mason (1987) estimated additional production of 1 009 000 adults. This total divided by the area of the three BLDP channels provides a production estimate of 8.55 adults/m² whereas the SEP biostandard is 10.8 adults/m². Earlier I discussed several reasons why 1 009 000 may be optimistic, but even given this number the current SEP biostandard is too high.

On the Fraser, the biostandards are even more difficult to understand. I have used the adjusted adult production numbers from Table 4. Based on the three Fraser River experiences the average production rate would be 5.15 adults/m². The SEP biostandard is 4.7 times that number and twice the Weaver Creek results.

SEP staff told me that the sockeye channel biostandards were derived by taking observed fry per square metre and multiplying by literature values for average fry to smolt and smolt to adult survivals for Fraser and non-Fraser stocks. This means that the observed fry production from Gates and Nadina was multiplied by average Fraser River survivals to produce expected adult production. This method of calculation overestimates production for Gates, Nadina, and all three Babine

TABLE 5. Observed production from existing channels and SEP biostandards.

Project	Total area (m ²)	Adult production	Adults (no./m ²)		Ratio SEP/observed
			Observed	SEP biostandard	
Fulton I	11 426				
Pinkut	33 442				
Fulton II	73 154				
BLDP total	118 022	1 009 000	8.55	10.8	1.26
Weaver	17 429	209 713	12.03	24.2	2.01
Gates	10 995	12 134	1.10	24.2	21.93
Nadina	18 131	17 703	0.98	24.2	24.79
All Fraser	46 555	239 550	5.15	24.2	4.70

channels. This method says that Gates and Nadina were great successes. The SEP biostandards were used in estimated anticipated benefit:cost ratios for the Chilko and Horsefly channels, therefore overestimating the anticipated benefits by a factor of 4. Thus the results of the adult evaluation have not been used in the planning criteria for subsequent channels.

Providing for performance evaluation of new projects

A second area where there appears to be a failure to learn from experience is in planning for evaluation. All previous channels appear to produce fry successfully; when problems occur they are either in smolt to adult survival (the BLDP case) or at some unknown life history stage after the fry (Gates and Nadina). If we examine the Chilko and Horsefly channels we find little evidence that this lesson has been absorbed.

First and foremost, both of these channels have been built in very successful existing sockeye runs (the 1989 adult production from the Horsefly was over 10 000 000). Even assuming that the Horsefly channel is as successful as planned, it would produce 200 000 to 300 000 adults, a level of production that would likely be indistinguishable from the natural production. Similar problems are found at Chilko, where the run is not quite as large, but does return in the millions, and is building. Therefore, if density-dependent smolt-adult survival is a problem in these systems (as it was in the Babine), these channels will probably never make any contribution, and furthermore, they can never be evaluated.

Not only will the adult production be impossible to evaluate, but because both the two new channels use intake water from streams with existing sockeye spawners, there will be naturally produced fry entering the channels, which will make it difficult to assess even egg to fry survival. I was told by several SEP biologists that evaluation of these facilities was not considered, and other biologists expressed great concern about the decision and design process for these two channels.

Discussion

Let us now return to the "big" question: do fisheries management agencies learn by experience? The examination of spawning channels shows that at one level the answer is yes. For operational decisions, the system works; decisions are monitored, the monitoring data evaluated, and the evaluation used in modifying subsequent decisions. Nevertheless, learning is slow. Considerable effort is still being expended on gravel cleaning methods, and there is lively debate about the appropriate spawner densities in channels.

At the second level — the decisions about when, where, and if more channels should be built — the picture is not nearly so

clear. The results of the evaluations of the BLDP and IPSFC channels indicate that the success of a project is quite unpredictable. Attempts to increase fry numbers beyond historical levels (as in the Babine) may well encounter some form of density-dependent limit at later life history stages.

An examination of the decisions of DFO to build subsequent channels leads to the conclusion that the results of previous evaluations were not used to any great extent. The evaluation design of the Chilko and Horsefly channels shows little if any learning from past experience. I was told by several biologists that the decisions to build these two channels were driven by political and engineering concerns — the biologists were disregarded.

Perhaps the most useful lesson from examining the history of spawning channels for sockeye is the long time required for evaluation. It was nearly 20 yr before sufficient data were accumulated to enable an examination of the success of the BLDP channels to produce adult sockeye. Even after 25 yr, no clear answer has emerged. Given the natural variability and change common to most fisheries systems, evaluation is likely to take a very long time. Decision makers desiring a quick answer need to understand that it may take 20–40 yr before answers are available, and even then they may be ambiguous.

One of the most striking features of the evaluation history of the Babine projects is how long it took to ask if adult production was actually increased! Although consideration of the projects began in the early 1960s, and the channels came on-line in the late 1960s, it was not until 1987 (West and Mason 1987) that anyone actually directly addressed the question of increased adult production. For many years it appears that adult production was simply not of interest. The following quotation from Ginetz (1977, p. 125) reflects this apparent lack of interest of adult production: "The Babine Development Project was initiated on the basis of the following premises: (1) that the main basin of Babine Lake was underutilized and could support additional sockeye fry, (2) that these additional sockeye fry could be produced in artificial spawning channels and in natural streams with regulated flow, and (3) that the channel fry so produced are comparable to naturally produced fry in their ability to survive to the adult stage."

There is no mention in this quote that increased fry and smolt production might not lead to increased adult production. In his subsequent discussion, Ginetz (1977) argued that the evidence supports the three assumptions and that therefore the Babine Development was a success. Admittedly by 1977 there were few adult return years, but Ginetz never mentioned it. However, Peterman (1978) was able to look at adult production from the Babine system and concluded that smolt to adult survival

declined as smolt numbers increased. These data were available to SEP biologists at the time but they did not choose to use them.

The same studious avoidance of adult production can be found in West (1978), Cooper (1977), and the unpublished consultant's report of 1985. While it did take 10 yr to accumulate sufficient brood year returns for evaluation, I believe the avoidance of consideration of adult production reflects "goal displacement" (Dowell and Wange 1986): the substitution of intermediate goals (fry production) for overall goals (adult production). The channel operators first and foremost want their channel to put fry out the gates of the channel. That is what they can measure, and so long as the channel can put out fry, their job is successful. A channel manager cannot assure that the fry find enough to eat in the lake, or avoid predators in the lake. Therefore there is a tendency to want to call a channel that produces fry a success. This rationalization is a natural phenomenon found throughout human behavior — the overall goal (adult production) is displaced by a simpler, more measurable goal (fry production).

The decisions to build the Chilko and Horsefly spawning channels might appear to indicate almost no learning from past experience about the success of spawning channels on the Fraser River. There is, however, another way to view this apparent lack of learning. The Pacific Salmon Treaty between the U.S. and Canada (ratified in 1985) set a ceiling on the U.S. catch of Fraser River sockeye. Previously under the IPSFC, the U.S. had been guaranteed half of the Fraser sockeye catch taken in convention waters. Canada had argued that it would not go ahead with enhancement activities on the Fraser so long as half of the benefits would accrue to the U.S.

Canada had promised its fishermen that if they made some short-term sacrifices, the U.S./Canada treaty would provide long-term benefits because of Fraser River enhancement. Having signed the treaty, DFO needed to show some enhancement on the Fraser. The Chilko and Horsefly channels are the major part of this enhancement. When DFO took over from the IPSFC, it was clear that spawners and fry production had been limited in many parts of the Fraser. There are few technologies available to enhance sockeye, and the Weaver Creek channel was successful. SEP staff argued that spawning channels were the best available artificial technology to enhance Fraser sockeye. I believe this is consistent with the evaluations.

Thus not only is the evaluation of spawning channels often ambiguous, the evaluation of the evaluation is equally ambiguous. On the one hand the biostandards used in planning do not reflect the results of evaluation. On the other hand, SEP now recognizes that part of its mandate is to enhance the Fraser, and the results of the evaluations do indicate that the spawning channels for sockeye are probably the best technology available. The only alternative sockeye technology used in British Columbia is lake enrichment, which has been used to increase juvenile growth in coastal lakes and is untried in the Fraser basin.

Has SEP learned from experience? The answer is yes, and perhaps no. Learning is most effective when goals are clearly defined, experiments can be conducted rapidly, and evaluation staff and decision makers are closely integrated. Decisions involving design and operation of spawning channels meet these conditions quite well, while the planning for further SEP activities does not.

The work on spawning channels described in this paper is part of a large project looking at other SEP technologies, and we are now examining learning in hatchery production for

(*O. tshawytscha*) and coho salmon (*O. kisutch*) and in lake enrichment for sockeye salmon. This is work currently in progress.

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Variability of Diel Vertical Migration in the Marine Planktonic Copepod *Pseudocalanus newmani* in Relation to Its Predators¹

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We report results of a 3-yr field study of the vertical distributions and diel vertical migration (DVM) of *Pseudocalanus newmani* in the central basin of Dabob Bay, Washington, USA. Our results include two novel findings. First, a statistically significant relationship exists between strength of DVM in *P. newmani* and the potential predation impact of its planktonic invertebrate predators. Second, a strong "normal" DVM (up at night, down during the day), unique for *P. newmani* in 5 yr of sampling at this locale, occurred at a time when the zooplanktivorous fish *Ammodytes hexapterus* was unusually abundant and preying on the copepod; this DVM may have been induced by the fish. DVM behavior of *P. newmani* was highly variable, with changes in behavior commonly occurring on a time scale of weeks; in one case the copepod switched from a normal migration pattern to a reverse migration pattern (down at night, up during the day) in less than 5 wk. These observations, combined with those of previous research, indicate that *P. newmani* has an exceptionally diverse repertoire of migration behavior, any particular expression of which is most likely manifested by individual copepods exercising phenotypic behavioral plasticity in response to potential predation.

Voici le compte rendu d'une étude sur le terrain ayant duré 3 ans, et portant sur la répartition verticale ainsi que la migration verticale nyctémérale du *Pseudocalanus newmani*, dans le bassin central de Dabob Bay, Washington, É.-U. Les résultats obtenus mènent à deux conclusions inédites. D'une part, il existe une relation importante, sur le plan statistique, entre l'intensité des migrations verticales nyctémérales chez le *P. newmani* et la prédation éventuelle exercée sur ce copépode par des invertébrés planctoniques. D'une part, une forte migration verticale nyctémérale, que l'on peut qualifier de «normale» (ascendante la nuit et descendante le jour) mais unique chez le *P. newmani* en 5 ans d'échantillonnage à cet endroit, s'est produite au cours d'une période où l'espèce zooplanctonophage *Ammodytes hexapterus* était particulièrement abondante et consommait de ce copépode; cette forte migration peut être imputable à ce poisson. Le comportement du *P. newmani* présentait de grandes variations, des modifications du comportement habituel s'étalant couramment sur des semaines; dans un cas, le copépode a inversé en moins de 5 sem son comportement migratoire normal (descendant la nuit et remontant le jour). Ces observations confirment celles d'une étude antérieure et indiquent que le *P. newmani* présente un répertoire extrêmement diversifié de comportements migratoires; en effet, tout copépode soumis à une prédation éventuelle démontre une plasticité de comportement phénotypique qui se manifestera très probablement de façon particulière selon les individus.

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Of the several hypotheses advanced to explain the adaptive value of diel vertical migration (DVM) behavior in zooplankton (Kerfoot 1985; Lampert 1989), evidence implicating predator evasion has recently accumulated from theoretical studies (Frost 1988; Gabriel and Thomas 1988; Mangel and Clark 1988), field observations (see brief review by Bollens and Frost 1989a; Levy 1990; Stirling et al. 1990; Bollens et al. 1992), and experimental manipulations of predators (Bollens and Frost 1989b, 1991; Dawidowicz et al. 1990; Leibold 1990; Neill 1990; Tjossem 1990; Ringelberg 1991). The DVM behavior of the marine planktonic copepod *Pseudocalanus newmani* Frost has played a particularly prominent role in the furtherance of the predator evasion hypothesis. Ohman et al. (1983) and Ohman (1990) inferred that 'reverse' DVM (at depth during the night, near the surface at day) in adult

female *P. newmani* in the deep, central basin of Dabob Bay (Washington) was a means of avoiding planktonic invertebrate predators that forage nocturnally in the surface layer. Further, "normal" DVM behavior (near the surface at night, at depth during the day) in *P. newmani*, not observed at the deep station, but occurring commonly at a nearby shallower station with a relatively depauperate assemblage of invertebrate predators, was suggested to be a means of avoiding zooplanktivorous fish that forage diurnally in the surface layer (Ohman 1990). With respect to the reverse migration of *P. newmani* at the deep station, Ohman (1990, fig. 12) presented field data relating the proportion of the population residing at depth at night during midsummer to the abundance of invertebrate predators above this depth at night; the relationship, while suggestive, was not, however, statistically significant.

Here, we report results of a 3-yr field study of the vertical distributions and DVM behavior of *P. newmani* in the deep, central basin of Dabob Bay and address the issue of how predators might induce changes in its DVM behavior.

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Materials and Methods

The field site was the deep (193 m), central basin of Dabob Bay, Washington, USA (47°45'N, 122°50'W). Twelve cruises were undertaken between 1985 and 1987: 25–26 April 1985, 25–26 June 1985, 20–22 August 1985, 8–15 October 1985, 6–8 May 1986, 9–11 June 1986, 5–6 August 1986, 13–15 August 1986, 10–12 June 1987, 15–17 July 1987, 12–14 August 1987, and 20–21 October 1987. During 1985 and 1986 the vertical distribution of adult female *P. newmani* was determined using a vertically hauled, multiple sample, closing net (1.0 m², 333- μ m mesh) described by Frost (1988). On these cruises the following six discrete depth strata were sampled: 0–10, 10–25, 25–50, 50–75, 75–125, and 125–175 m. In May 1985 the two surface strata were combined into a single stratum of 0–25 m. During the 1987 cruises, we used a vertically hauled, single sample, closing net (1.0 m diameter, 216- μ m mesh) described by Miller et al. (1984) and sampled 0–25, 25–50, and 50–175 m strata. On all 12 cruises, duplicate samples were collected near noon and midnight on each of two consecutive days. Abundance of adult females of *P. newmani* in preserved samples was determined from the mean abundance in replicate subsamples taken with a Folsom splitter, Stempel pipette, or automatic pipette.

For each of our 12 cruises, as well as for 15 dates reported in Ohman et al. (1983) and Ohman (1990; data kindly provided by the author), we calculated two separate indices of DVM in *P. newmani*. First, we calculated the weighted mean depth (WMD) of adult females for each daytime and nighttime vertical series of samples, where $WMD = (\sum n_i d_i) / \sum n_i$ and n_i is abundance (number per cubic metre) at depth d_i , taken to be the midpoint of each depth stratum. The amplitude of migration (ΔZ) was then calculated as the difference between the mean daytime WMD and the mean nighttime WMD, with positive values indicating normal DVM and negative values indicating reverse DVM. Each ΔZ value was tested for statistical significance using a *t*-test (two-tailed) to compare mean daytime and nighttime WMDs. Second, we calculated the proportion of adult female *P. newmani* migrating on a diel cycle across a reference depth of 25 m by calculating the difference between the mean proportion of the population above 25 m at midnight and the mean proportion of the population above 25 m at midday (i.e. *V* as described by Bollens and Frost 1989a). Although the choice of reference depth for this index is somewhat subjective, 25 m is the depth across which *P. newmani* most commonly migrates when exhibiting DVM at this locale (Ohman et al. 1983; Ohman 1990; this study).

Abundances of planktonic invertebrate predators in the upper 25 m at night were determined from the same samples used to determine the vertical distributions of *P. newmani*. We enumerated the carnivorous copepod *Euchaeta elongata* (copepodid IV, copepodid V, and adult female stages), the chaetognath *Sagitta elegans*, and the euphausiids *Euphausia pacifica* and *Thysanoessa longipes* (>10.5 mm, i.e. late juveniles and adults). These taxa represent the major invertebrate predators of *P. newmani* at this locale (Ohman 1986, 1990). To estimate the potential mortality imposed by the predator populations, we determined maximal potential predation rates (number of female *P. newmani*·d⁻¹) by multiplying predator density by experimentally derived maximal ingestion rates obtained from the literature. These maximal ingestion rates are 4.52, 16.1, and 18.7 female *P. newmani*·predator⁻¹·d⁻¹ at 8°C for copepodid IV, copepodid V, and adult female *E. elongata*, respectively (Yen 1983), 2.26 female *P. newmani*·predator⁻¹·d⁻¹ at

8°C for *E. pacifica* (Ohman 1984), and 2.20 female *P. newmani*·predator⁻¹·d⁻¹ at 13°C for *S. elegans* (Reeve 1980). Maximal ingestion rate for *T. longipes*, which was always much less abundant than *E. pacifica*, was assumed to be equal to that for *E. pacifica*. These maximal predation rates were then adjusted for seasonal changes in temperature by applying the temperature predicted to occur in the middle of the 25–0 m stratum on each date (minimum of 8°C on February 1, maximum of 14°C on August 1; Ohman 1986), assuming a Q_{10} of 2.0.

On most dates, we obtained estimates of light attenuation in the surface layer from measurements of Secchi depth or vertical profiles of submarine irradiance using a LiCor 190SB underwater quantum sensor. Phytoplankton standing stock was measured as concentration of chlorophyll *a* in samples collected at five to seven depths in the upper 30 m (see Frost 1988; Ohman 1990). Sampling of planktivorous fish and gut content analysis of fish were done as described in Bollens and Frost (1989a).

Results and Discussion

Pseudocalanus newmani exhibited diverse migration behavior (Table 1). Considering only those sampling dates with sufficient replication for statistical testing (20/27 cases), *P. newmani* usually did not migrate (i.e. mean daytime WMD was not significantly different from mean nighttime WMD on 11 of 20 dates). Reverse DVM occurred on eight dates, thus doubling the number of cases reported by Ohman (1990). Normal migration was observed only once during 6–8 May 1986 (Table 1).

Simple linear regression (functional regression, Ricker 1973) was used to examine the dependence of the strength of DVM in *P. newmani* (*V*) on the potential predation rate of invertebrate predators (Fig. 1). For this analysis, we combined data from our 11 cruises with that from 11 cruises reported in Ohman (1990) in which concurrent sampling of *P. newmani* and its invertebrate predators was undertaken. The strength of DVM in *P. newmani* showed a highly significant (negative) dependence on potential predation rate regardless of which index of migration was used as the dependent variable (for *V*: $Y = 17.10 - 0.075X$, $r = -0.722$, $n = 22$, $P < 0.01$; for ΔZ : $Y = 9.194 - 0.044X$, $r = -0.762$, $n = 22$, $P < 0.01$). Moreover, both of these migration indexes also showed significant (negative) dependences on the absolute abundance of invertebrate predators (for *V*: $Y = 24.30 - 0.486X$, $r = -0.595$, $n = 22$, $P < 0.01$; for ΔZ : $Y = 13.36 - 0.281X$, $r = -0.465$, $n = 22$, $P < 0.05$).

The single instance of normal vertical migration (6–8 May 1986: Table 1; Fig. 2A), unique for *P. newmani* at this locale in 5 yr of sampling (Ohman 1990; this study; B. W. Frost, unpubl. data), occurred at a time when late larval and juvenile *Ammodytes hexapterus* (Pacific sandlance) were unusually abundant (Bollens and Frost 1989a). This fish is a known zooplanktivore (Hart 1973) and was actively feeding on adult female *P. newmani* at this time (Table 2). Gut content analyses were also done on five other species of fish collected at the station on this date (Bollens and Frost 1989a). Guts of four of these species (*Oncorhynchus keta* (chum salmon), *Gasterosteus aculeatus* (threespine stickleback), *Clupea harengus* (Pacific herring), and an unidentified osmerid (smelt)) contained no *P. newmani*, while guts of the fifth species (*Oncorhynchus gorbuscha* (pink salmon)) contained only trace amounts of *P. newmani* (<1% of gut contents by number or weight). Indeed, in 3 yr of regular trawl sampling of pelagic

TABLE 1. Daytime and nighttime weighted mean depths (WMDs) and summary statistics for adult female *P. newmani* at the central, deep station of Dabob Bay. 1973–80 calculations generated from data in Ohman et al. (1983) and Ohman (1990); 1985–87 calculations generated from unpublished data available from the authors upon request. See text for details regarding calculations. Negative (positive) values of ΔZ and V indicate reverse (normal) DVM. Probability that mean nighttime and mean daytime WMDs different by chance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns = not significant. Blank cells indicate samples not collected or summary statistics not applicable given lack of replication on that date.

Dates	Daytime WMDs (m)					Nighttime WMDs (m)					Day vs. night comparisons		
	1	2	3	4	Mean	1	2	3	4	Mean	ΔZ (m)	<i>t</i> -statistic	<i>V</i> (%)
3–4 Aug. 1973	38.4	34.0			36.2	72.8	85.0			78.9	–42.7	6.58*	–56.9
1–2 Aug. 1978	36.4					56.1					–19.7		+4.8
24 Aug. 1978	23.2					51.7					–28.5		–46.7
28–30 Sept. 1978	19.2	24.9			22.1	23.3	18.0			20.7	+1.4	0.36ns	+5.0
11–12 Jan. 1979	11.5					13.9					–2.4		–1.6
15–16 Mar. 1979	27.8					25.0					+2.8		+2.5
11–12 Apr. 1979	21.2					20.2					+1.0		+5.1
4–7 June 1979	28.6	24.2			26.4	28.5	51.9			40.2	–13.8	1.16ns	–24.6
10–11 July 1979	32.3					44.9					–12.6		–34.2
25–31 July 1979	28.1	30.8			29.5	75.2	72.3			73.8	–44.3	22.35**	–67.2
14 Aug. 1979	14.1	16.9			15.5	41.8	36.9			39.4	–23.9	8.50*	–66.7
5–7 Dec. 1979	24.9	24.0	23.2		24.0	24.9	25.1			25.0	–1.0	1.57ns	+11.1
9–10 July 1980	11.0					16.2					–5.2		–24.2
26–28 July 1980	21.1	21.9			21.5	30.8	25.4			28.1	–6.6	2.42ns	–36.0
16–19 Aug. 1980	21.6	26.4			24.0	54.1	48.2			51.2	–27.2	7.15*	–43.7
25–26 Apr. 1985	31.8	30.4	34.9	30.0	31.8	30.2	29.1	29.2	26.2	28.7	+3.1	2.21ns	+10.9
25–26 June 1985	15.1	13.7	18.6	17.4	16.2	17.2	19.0	13.7	14.4	16.1	+0.1	0.06ns	–25.9
20–22 Aug. 1985	30.6	29.2	26.9	26.0	28.2	46.1	55.3	33.7	48.3	45.9	–17.7	3.83**	–26.8
8–15 Oct. 1985	12.8	15.6	7.9	7.0	10.8	10.9	10.9	11.5	9.1	10.6	+0.2	0.10ns	+2.8
6–8 May 1986	44.3	42.0	37.8	40.3	41.1	19.7	13.9	15.5	19.2	17.1	+24.0	12.19***	+53.6
9–11 June 1986	9.8	9.0	13.1	10.3	10.6	19.5	22.0	13.2	13.8	17.1	–6.5	2.78*	–13.3
5–6 Aug. 1986	28.3	35.9	49.9	59.5	43.4	36.3	37.9	40.2	51.5	41.5	+1.9	0.24ns	–4.9
8–15 Oct. 1986	9.6	12.5	13.0	16.0	12.8	10.3	9.3	14.8	10.3	11.2	+1.6	0.89ns	+5.3
10–11 June 1987	19.8	21.9	22.6	26.6	22.7	21.8	21.7	22.1	20.6	21.6	+1.1	0.75ns	+4.3
14–17 July 1987	33.4	31.4	29.7	26.4	30.2	50.8	48.0	58.8	47.5	51.3	–21.1	7.03***	–22.5
12–14 Aug. 1987	20.5	31.1	34.1	27.2	28.2	51.8	42.9	40.1	43.3	44.5	–16.3	4.21**	–33.3
20–21 Oct. 1987	27.5	45.0	56.3	54.2	45.8	30.6	25.4	39.3	42.8	34.5	+11.3	1.47ns	+18.2

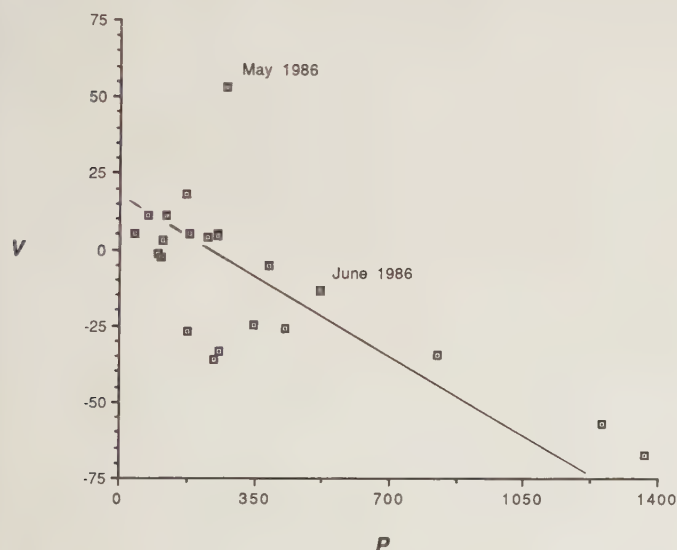


FIG. 1. Linear regression of the dependence of the strength of diel vertical migration (V) of adult female *P. newmani* in the deep, central basin of Dabob Bay on potential predation rate (P , number of female *P. newmani* removed $\cdot m^{-3} \cdot d^{-1}$) ($Y = 17.10 - 0.075X$, $r = -0.722$, $n = 22$, $P < 0.01$). Line fitted by functional regression (Ricker 1973).

fish and analyses of their gut contents at this station (e.g. Bollens and Frost 1989a and unpublished data for 1987), *P. newmani* rarely exceeded these trace levels in the diets of fish and never was as dominant a dietary item as in

A. hexapterus in May 1986. The unique normal DVM exhibited by *P. newmani* during 6–8 May 1986 may have been induced by its unusually abundant predator, *A. hexapterus*.

For 21 sampling dates, we had estimates of light attenuation coefficient and phytoplankton standing stock (as milligrams of chlorophyll *a* per square metre, integrated over the upper 30 m). We found no significant correlation between either of these and either measure of strength of DVM in *P. newmani* (all correlation coefficients positive, but < 0.3 , $N = 21$).

These results suggest that DVM in *P. newmani* at this locale was largely a response to predation pressure from invertebrates and thus corroborate and lend a strong statistical basis to findings previously reported by Ohman et al. (1983) and Ohman (1990). Our results are novel, however, in indicating that the behavioral repertoire of *P. newmani* at this locale also includes normal DVM behavior. Thus, while no migration or reverse DVM was the most common behavioral mode in *P. newmani* at this locale (Table 1), with the latter behavior hypothesized to be induced by invertebrate predators, the normal DVM suggests an occasional inducing role for visual predators such as zooplanktivorous fish. Levy (1990) reported similar diversity of migration behavior in a freshwater cladoceran (*Bosmina*) and also suggested that different migration patterns were responses to presence and behavior of specific types of zooplanktivores.

Vertical migration behavior of *P. newmani* at this locale was not only highly variable, but could also sometimes change rather rapidly, e.g. from strong normal DVM behavior in May of 1986 to weak reverse migration just 5 wk later (Table 1; Fig. 2).

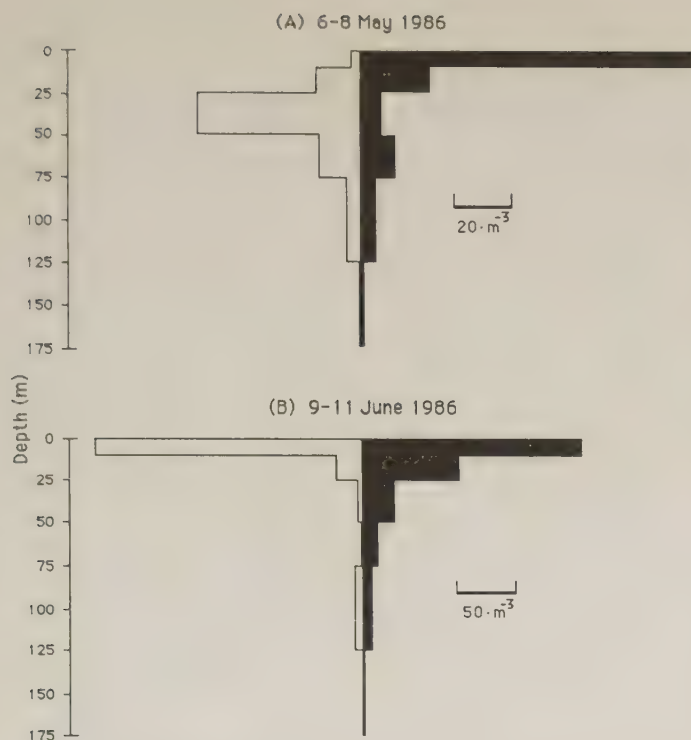


FIG. 2. Vertical distribution of adult female *P. newmani* in the central, deep basin of Dabob Bay. Each daytime (white) and nighttime (black) vertical distribution is the mean of four vertical series of samples collected in pairs near noon and midnight on each of two consecutive days.

TABLE 2. Composition of the diet, as percent of total numbers of different prey types, in seven juvenile (48–55 mm standard length) *A. hexapterus* collected at the deep, central station in Dabob Bay, on 1 May 1986. "Other" consisted of miscellaneous crustaceans, largely calanoid copepods and larval euphausiids. See Bollens and Frost (1989a) for details of collection and analysis of stomach contents.

Prey category	%
<i>Calanus pacificus</i>	11
<i>Oithona similis</i>	27
<i>Pseudocalanus newmani</i>	31
Other	31

Although we have no direct measurements of advection with which to address the possibility of substitution of behavioral types at this station, we believe that the change in behavior was not due to exchange of populations within Dabob Bay, for in concurrent sampling at the shallow station, *P. newmani* exhibited a weak (statistically nonsignificant) normal DVM during 9–10 June 1986 (B. W. Frost, unpubl. data). Ohman (1990) also observed differences in migration behavior of *P. newmani* sampled concurrently at the two stations.

The rapid shift of migration behavior at the deep station has implications for the mechanism by which predation might induce such changes. For instance, selective predation could impose differential mortality on genetically distinct behavioral types within the prey population and thus, over time, cause the observed changes in DVM behavior. Alternatively, individual copepods could simply be exercising phenotypic plasticity in their DVM behavior and thus effect very rapid changes in migration patterns.

The possibility of a population – genetic level behavioral response is supported by electrophoretic studies of freshwater

cladocerans showing different genotypes of the same species to exhibit differences in DVM behavior (Weider 1984) and phototaxis (Dumont et al. 1985; De Meester and Dumont 1988, 1990). However, the change in behavior in *P. newmani* from normal migration in May 1986 to reverse migration in June 1986 occurred during a period of substantial increase in abundance of adult females of *P. newmani* (from 2497 ± 171 to $4380 \pm 456 \cdot m^{-2}$ (mean $\pm$ SE), which would require the hypothesized mechanism of selective predation to inflict very much greater mortality on normal migrators in order for the reverse migrators to grow rapidly and become dominant in only 5 wk. However, the occurrence during this period of moderate to high potential invertebrate predation (Fig. 1), on the one hand, and an unusually high abundance of planktivorous fish (Bollens and Frost 1989a) actively feeding on *P. newmani* (Table 2), on the other hand, suggests that both behavioral types would be expected to experience substantial mortality, and thus a very much greater rate of mortality on normal migrators during this period seems unlikely.

Given this probable limitation on differential mortality to cause such rapid shift in migration behavior, the more likely explanation for the change we observed in DVM behavior in *P. newmani* is phenotypic behavioral flexibility of individual copepods. This interpretation of our field results is consistent with recent experimental findings showing individual phenotypic flexibility in DVM behavior in two other species of copepods (Bollens and Frost 1989b, 1991; Neill 1990). However, while such a hypothesis is suggested by our field data on *P. newmani*, it will ultimately need to be tested experimentally.

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Assessment of Activity-Specific Metabolism of Aquatic Organisms: An Improved System

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Davies, R. W., F. J. Wrona, and V. Kalarani. 1992. Assessment of activity-specific metabolism of aquatic organisms: an improved system. *Can. J. Fish. Aquat. Sci.* 49: 1142–1148.

An improved flow-through respirometer capable of assessing activity-specific metabolism of aquatic organisms is presented and assessed. The system is highly sensitive and versatile, since it continuously monitors and records activity-specific oxygen consumption readings for periods up to 72 h and is capable of detecting differences in metabolism of individual specimens of similar weight. Using this system, we demonstrated individual variation and intraspecific differences in metabolism between two size classes of the freshwater leech *Nepheleopsis obscura* and interspecific differences between *N. obscura* and another freshwater leech, *Erpobdella montezuma*, and compared these findings with the metabolism of the amphipod *Hyaella montezuma*.

On a présenté et évalué un respiromètre à circulation continue capable de mesurer le métabolisme d'organismes aquatiques pendant certaines activités. Cet appareil est très sensible et présente de nombreuses possibilités, car il assure une surveillance continue et enregistre les lectures de la consommation d'oxygène associée aux diverses activités pour des périodes allant jusqu'à 72 h; il peut également détecter des différences de métabolisme entre des spécimens de poids équivalents. À l'aide de cet appareil, on a enregistré des variations individuelles et intraspécifiques dans le métabolisme entre deux catégories de taille, chez la sangsue d'eau douce *Nepheleopsis obscura*, et des différences interspécifiques entre *N. obscura* et une autre sangsue d'eau douce, *Erpobdella montezuma*; on fait également la comparaison de ces résultats avec le métabolisme de l'amphipode *Hyaella montezuma*.

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Often the most difficult and time-consuming information to collect in bioenergetic studies of aquatic organisms is accurate, repeatable measures of metabolism. Oxygen consumption is the most frequently used method to measure metabolism, and to this end, numerous respirometer systems have been developed, each having their respective advantages and disadvantages (reviewed in Lampert 1984; Wrona and Davies 1984). Respirometers can be broadly classified as closed (static) or open (flow-through) systems, the latter being developed to overcome a number of serious limitations and biases associated with closed systems. These include declining oxygen availability in the experimental chambers, physiological stress from increasing metabolite accumulation (Kamler 1969; Gnaiger 1983a), disturbance from mechanical agitation devices often used to eliminate gas stratification (e.g. Warburg and Gilson respirometers) (Rueger et al. 1969; Nagell 1975; Gnaiger 1983b), and the use of lights for taking readings required in some volumetric methods. In addition, most respirometers (static and flow-through) are incapable of correlating oxygen uptake with defined activity patterns, further limiting their usefulness in bioenergetic studies (Wrona and Davies 1984).

Although many of the problems outlined above were overcome in the flow-through respirometer designed by Wrona and Davies (1984), their system was not amenable for use in long-term monitoring of metabolism (i.e. >12 h), imposed practical difficulties in obtaining large numbers of replicate observations, and was limited in its ability to detect small activity-related changes in oxygen uptake, particularly of individuals with small body size.

This paper describes improvements made to the original Wrona and Davies (1984) system and presents the results of four experiments that test the new system's ability to detect differences in metabolism of individuals of similar body size, examines potential biases resulting from bacterial colonization during long-term (72 h) monitoring of metabolism, examines time-related differences in resting and active metabolism during long-term monitoring, and assesses intra- and interspecific differences in metabolism between two size classes of two freshwater leech species (*Nepheleopsis obscura* Verrill and *Erpobdella montezuma* Davies et al.) and one species of amphipod (*Hyaella montezuma* Cole and Watkins).

Materials and Methods

The respirometer system consists of four major components: the flow-through respiration chambers, an activity monitoring system, a temperature-controlled water circulation and filtration system, and a microcomputer data acquisition and control system (Fig. 1).

Respiration Chambers

Four chambers (three experimental and one control) constitute the respirometer system. Each respiration chamber (RC) is a modified Hamilton 1000 gas-tight glass syringe, whose internal dimensions can be adjusted to accommodate different sizes of animals by moving the plunger position. As syringes of different volumes can be used, the system is very adaptable. Each syringe is further modified by drilling a hole through the stainless steel plunger and adding a Teflon tip, fitted with an O-ring

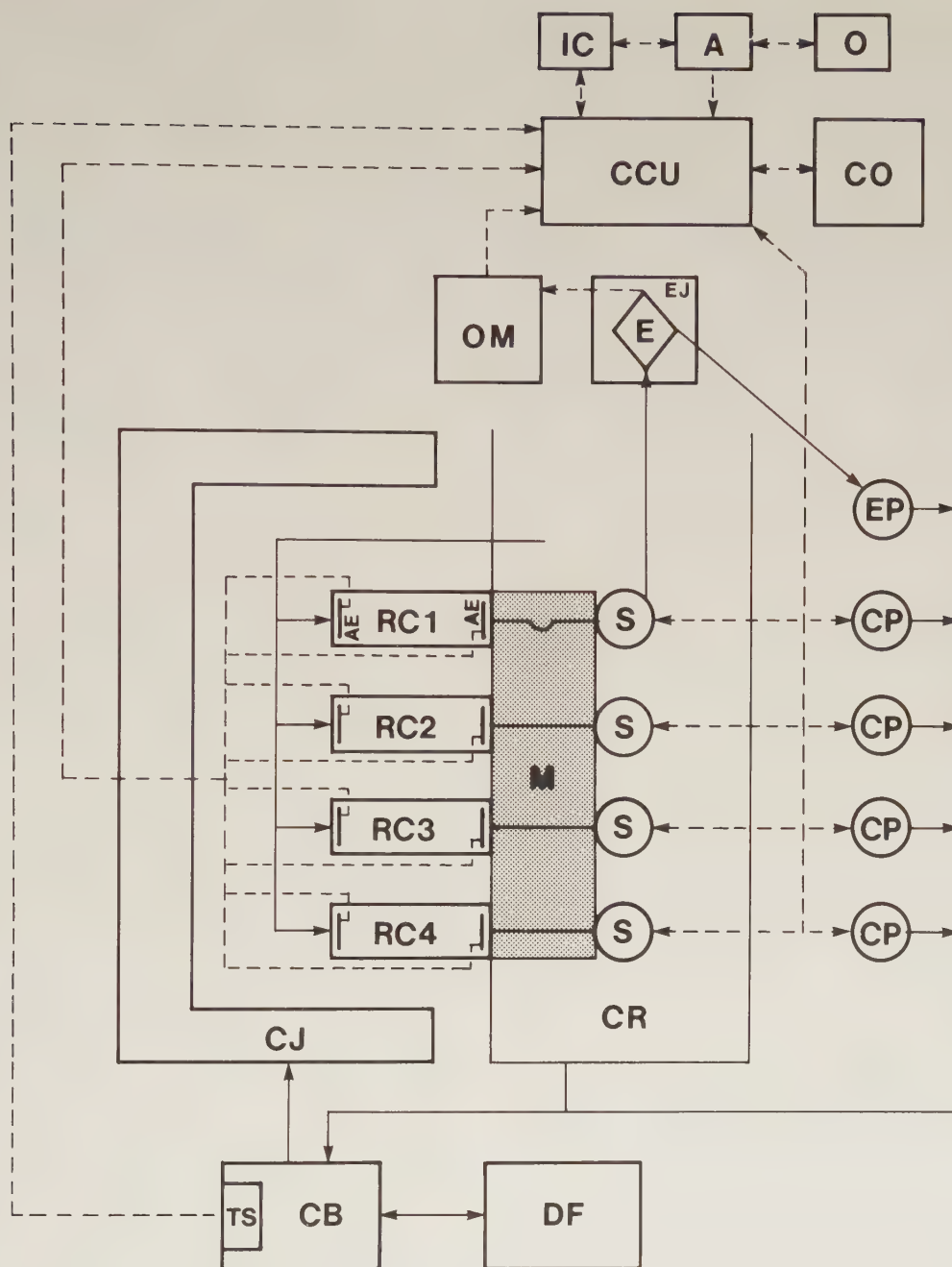


FIG. 1. Schematic diagram of the flow-through respirometer with measurement taken from RC1. CB, circulating bath; DF, Diatomite filter; CJ, cooling jacket; CR, cooling reservoir; RC1–RC4, respiration chambers; M, manifold block; S, solenoids; EP, electrode pump; CP, control pumps; E, electrode; EJ, electrode jacket; OM, oxygen meter; CCU, central control unit; CO, microcomputer; IC, impedance converter; A, preamplifier; O, oscilloscope; AE, activity electrode; TS, temperature sensor. Solid lines indicate the flow of water; broken lines indicate electrical connections.

large enough to accommodate a stainless steel inlet tube that also functions as the negative electrode for the impedance activity monitoring system. The threaded head of the syringe is screwed directly into a stainless steel plate (SSP) that is attached to the front of a teflon manifold (M) containing an array of internal channels (Fig. 2). Connected independently to each of the output channels of the manifold are four electronic three-way solenoid valves (S) (Lee Company model J-6468-87). Both the manifold and solenoid valves are submerged in a cooling reservoir (CR) through which experimental water circulates. The respiration chambers themselves are not submerged but are

surrounded by a cooling jacket (CJ) that thermostats them to the experimental temperature. Connections are made of stainless steel capillary tubes and Teflon tubes with Teflon/nylon adaptors fitted with O-rings to ensure gas- and water-tight connections.

Activity Monitoring System

A subcomponent of the respirometer system is the activity monitoring system. Activity of an organism in a respiration chamber is monitored using a U.F.I. (Biocom) model 2991 impedance converter (IC) connected to two electrodes (AE)

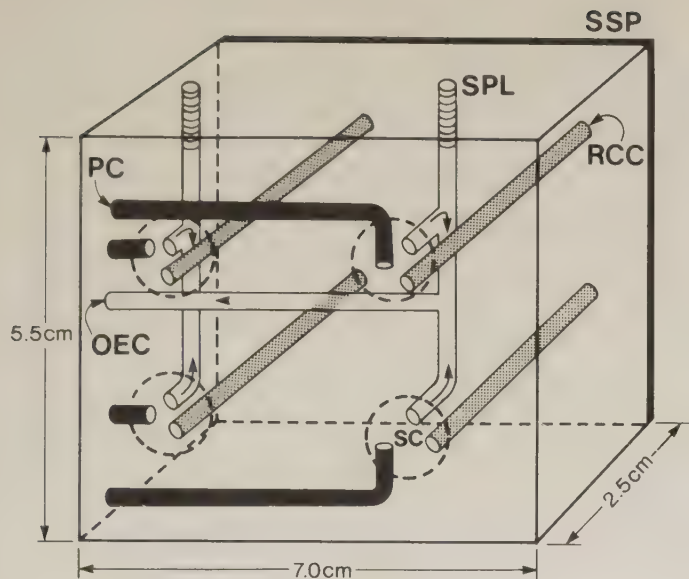


FIG. 2. Respirometer manifold showing details of the water flow. SPL, screw plugs; SC, solenoid connection; RCC, respiration chamber connection; PC, pump connection; OEC, oxygen electrode connection; SSP, stainless steel plate. Shaded, inflow; black, normal outflow; white, control outflow into oxygen meter.

made of stainless steel mesh disks (mesh diameter <0.5 mm), one placed at each end of a chamber. Activity by the animal results in a change in electrical impedance between the two electrodes, which in turn is detected by the impedance converter whose signal is amplified by a Grass P15 AC preamplifier (A). The output from the preamplifier is connected to the computer data acquisition system and/or is displayed directly on an oscilloscope (O).

Water Circulation and Filtration

Water of known temperature and oxygen concentration is produced by bubbling compressed air, oxygen, or other gas mixtures through airstones placed at the bottom of the Haake A82 refrigerated circulating bath (CB) and in the cooling reservoir containing the manifold and solenoid valves (Fig. 1). Water pumped from the circulating bath first enters a Plexiglas cooling jacket (CJ) that surrounds the respiration chambers. Water then exits the cooling jacket and enters a Radiometer oxygen electrode jacket (EJ) that thermostats the oxygen electrode (E) to the experimental temperature. Upon leaving the electrode jacket, water enters the cooling reservoir that bathes the manifold and solenoid valves and also becomes the input water source for the respiration chambers. A constant water level is maintained in the cooling reservoir, and overflow is returned back into the circulating bath for recirculation. Suspended particles and bacteria are filtered from the circulating water by a Magnum Diatomite filter (DF) that is connected to the circulating bath.

Water is drawn continuously through each respiration chamber by four peristaltic pumps (CP) (Pharmacia Peristaltic Pump P-1: flow range $0.6\text{--}500\text{ mL}\cdot\text{h}^{-1}$), each connected independently to one of the four solenoid valves. A fifth oxygen electrode pump (EP) sucks water through the oxygen electrode during the measurement of respiration, which is measured by the Strathkelvin oxygen meter (OM) (model 781b) in one chamber at a time. Hence, water flow from each respiration chamber can be directed to either the oxygen electrode (Radiometer

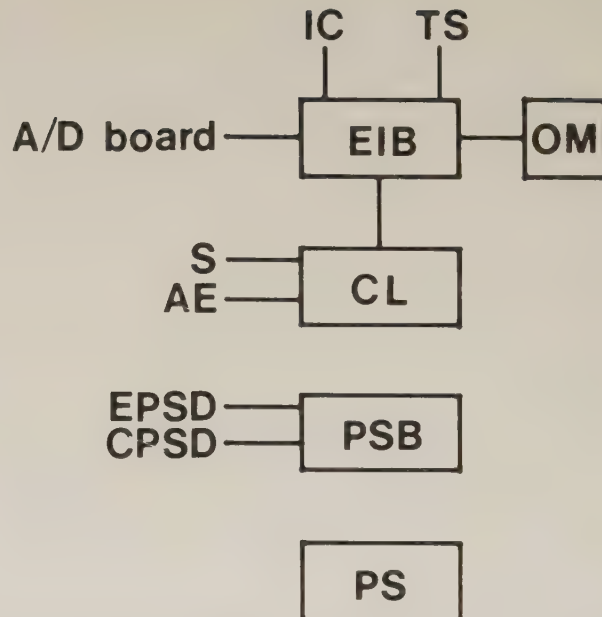


FIG. 3. Details of the central control unit. EIB, exterminator interface board; CL, control logic; PSB, pump speed board; PS, power supply; IC, impedance converter; TS, temperature sensor; OM, oxygen meter; S, solenoids; AE, activity electrode; EPSP, electrode pump speed board; CPSD, circulating pump speed board.

E5046 PO_2 electrode) or bypassed into the circulating bath. When oxygen readings are being recorded from a given respiration chamber, the circulation pump to that chamber is switched off and water flow is directed to the oxygen electrode by the solenoid valve. Water flow through all the peristaltic pumps eventually returns to the circulating bath for reoxygenation, filtration, and recirculation.

Connections between the manifold and the solenoid valves and the manifold and the peristaltic pumps are made as short as possible to reduce time lags associated with water leaving the respiration chambers and entering the oxygen probe for measurement.

Microcomputer Data Acquisition and Control System

Automation and control of the operation of the respirometer (i.e. the sequential turning on and off of the solenoid valves, data acquisition from the impedance system and oxygen meter, control of the sampling frequency, duration, and sampling regime) is achieved using the software package Labtech Notebook (Ver. 6.0), the ADALAB analog/digital data acquisition and control board, and an exterminator interface board (EIB) (Mandel Scientific Co. Ltd., Toronto). Outputs from the oxygen meter, impedance converter, peristaltic pumps, solenoids, activity electrodes, and temperature probe are connected directly to the EIB, which is housed within a central control unit (CCU). The EIB is connected directly to the ADALAB analog/digital board (A/D board), slotted within a Zenith 286 microcomputer (Fig. 3), and is capable of processing both analog and digital signals using three analog input and eight digital output channels. The analog inputs transmit information on (1) the water temperature from the temperature sensor (TS) in the Haake water bath, (2) the activity signal from the impedance converter and preamplifier, and (3) oxygen tension from the oxygen meter. The eight digital output lines control the on/off operation of the peristaltic pumps, the switching of the solenoid valves, and the switching of the activity channels. The

control logic (CL) connected directly to the EIB provides the switching of the activity electrodes and the solenoids.

In addition to the EIB, the CCU houses a custom-built pump speed board (PSB) containing two digital counters. One counter is connected directly to the speed-output line of the fifth peristaltic pump (electrode pump speed display (EPSD)) that draws water through the oxygen electrode, and the other counter is switched between the four remaining pumps (circulating pump speed display (CPSD)) connected to each of the respiration chambers. The CCU also houses a power supply (PS) which provides power to the control logic (+5 V), the pump speed counter (+12 V), and the solenoid valves (+12 V). The EIB is powered directly from the computer via the cable that links it to the A/D board.

Respirometer Operation

Oxygen uptake is calculated using the relationship

$$MO_2 = \frac{(P_cO_2 - P_eO_2)(\alpha O_2)(F)}{dwt}$$

where MO_2 = rate of oxygen consumption (micromoles per milligram per hour), P_cO_2 = partial pressure of oxygen in the water of control chamber (torr) (1 torr = 133.322 Pa), P_eO_2 = partial pressure of oxygen in the water of experimental chamber (torr), αO_2 = solubility coefficient of oxygen in water at a defined temperature (micromoles per litre per torr) from Dejours 1975), F = flow rate of water through the respiration chamber (litres per hour), and dwt = dry weight of individual (milligrams).

P_cO_2 and P_eO_2 are obtained when the water from the control respiration chamber and the three experimental respiration chambers reaches the oxygen electrode, respectively. The flow rate through the respiration chambers is adjusted so that a minimum of 5 torr difference is always obtained between P_cO_2 and P_eO_2 readings.

In a typical short-term run (<24 h) (e.g. Experiments I and IV below), the sampling regime alternates between the control and experimental chambers in the following sequence: control (20-min reading); experimental chamber 1 (40 min); control (20 min); experimental chamber 2 (40 min), etc. Data readings, including time (seconds), temperature (degrees Celsius), chamber number, partial pressure of oxygen (torr), and activity level, are taken once every second (i.e. a sampling frequency of 1.0 Hz) and are stored directly onto a hard disk drive for future analysis. Long-term runs (>24 h duration) (e.g. Experiments II and III below) follow the same sampling sequence except that the control and experimental chambers are now monitored for 20 and 60 min, respectively, the sampling frequency is altered to 0.25 Hz (i.e. once every 4 s), and each run is repeated six times for each 24-h period.

Data collected during the time lag for each chamber were not used. When actual oxygen uptake rates are calculated for a given sampling period, the average partial pressure of oxygen (P_cO_2) measured in the control chamber immediately preceding the particular experimental chamber is used. Oxygen consumption of the animal is then converted to a volumetric rate (at STP) using $VO_2 = MO_2 (22.414 \text{ L} \cdot \text{mol}^{-1})$, where VO_2 = rate of oxygen consumption (microlitres per milligram per hour). The difference between the active and resting VO_2 gives the aerobic scope.

Testing of the Respirometer System

Four experiments were carried out to validate and test the respirometer system using nonreproductive individuals of the

TABLE 1. Mean ($\pm$ SE) oxygen uptake rates ($\mu\text{L O}_2 \cdot \text{mg dwt}^{-1} \cdot \text{h}^{-1}$) and aerobic scope for three *N. obscura* of similar size ($n = 1000$ oxygen determinations).

<i>N. obscura</i> (mg dwt)	Resting	Active	Aerobic scope
50.1	0.92 $\pm$ 0.03	2.33 $\pm$ 0.02	1.41 $\pm$ 0.03
50.0	1.19 $\pm$ 0.02	3.47 $\pm$ 0.02	2.26 $\pm$ 0.02
50.2	1.47 $\pm$ 0.01	2.71 $\pm$ 0.01	1.25 $\pm$ 0.01

freshwater erpobdellid leeches *N. obscura* (from southern Alberta) and *E. montezuma* (from Arizona) and the freshwater amphipod *H. montezuma* (from Arizona). Two groups of *N. obscura*, one collected in May 1990 and which had survived winter (termed winter *N. obscura*) and the second collected as cocoons immediately after deposition in July 1990 and hatched in the laboratory at 20°C (termed summer *N. obscura*), were used. Dratnal and Davies (1990) showed that winter and summer *N. obscura* are significantly different in terms of growth rates, feeding rates, and life history traits. Animals were maintained separately for 3 wk in aerated habitat water at 20 $\pm$ 0.2°C, with constant light. *Nephelopsis obscura* and *E. montezuma* were fed every second day with *Tubifex tubifex* and *H. montezuma* juveniles, respectively, and *H. montezuma* was fed with algae. All animals were starved for 3 d prior to the start of the experiments.

Experiment I

Experiment I tested the ability of the system to detect differences in metabolism of individuals of similar body size. Three winter *N. obscura* of 265 mg wet weight (wwt) were individually acclimated to the test conditions in respiration chambers for 1 h and the partial pressure of oxygen in the control and the three experimental chambers measured for 3 h. The resting and active metabolic rates and the aerobic scope of each individual were measured by taking 1000 replicate readings.

Experiment II

Experiment II examined the potential biases resulting from bacterial colonization during long-term (72 h) monitoring of metabolism. Three winter *N. obscura* (750 mg wwt) were similarly acclimated for 1 h and oxygen changes monitored for 24 h. At the end of 24 h, the leech from experimental chamber 1 was removed, at the end of 48 h the leech from experimental chamber 2 was removed, and at the end of 72 h the leech from experimental chamber 3 was removed. In each case the mucus secreted by the leech was left in the respirometer chamber. Oxygen measurements were continued for a further 24 h. The oxygen meter was recalibrated every 24 h. For analysis, data collected at 24, 48, and 72 h are utilized. Student's *t*-test was used to test for time-related changes in P_cO_2 ($n = 860$ replicate readings) compared with respective P_eO_2 ($n = 260$ replicate readings).

Experiment III

Experiment III examined time-related differences in resting and active metabolism during long-term monitoring. Three winter *N. obscura* of the same size (750 mg wwt) were acclimated to the test conditions in the respiration chambers for 1 h and readings of resting and active respiration taken continuously for a period of 72 h. Two hundred replicate readings with the animal resting and another 200 replicate readings when active were taken separately for each individual after 1, 20, 24, 44, 48, and 68 h. A model I one-way ANOVA was used for testing time-related differences in average resting and active oxygen uptake rates and aerobic scope of the individuals.

TABLE 2. Mean ($\pm$ SE) dissolved oxygen (torr) in the control chamber (C) ($n = 260$ determinations) and experimental chambers (E_1 , E_2 , and E_3) ($n = 860$ determinations) containing *N. obscura* weighing 750 mg wwt after 24, 48, and 72 h. All experimental values are not significantly different from the respective control values ($P > 0.05$).

Time (h)	C	E_1	C	E_2	C	E_3
24	128.79 $\pm$ 0.18	128.07 $\pm$ 0.19	—	—	—	—
48	133.04 $\pm$ 0.27	132.02 $\pm$ 0.19	133.36 $\pm$ 0.19	133.02 $\pm$ 0.17	—	—
72	133.73 $\pm$ 0.17	133.22 $\pm$ 0.16	133.62 $\pm$ 0.16	133.02 $\pm$ 0.16	135.12 $\pm$ 0.07	134.59 $\pm$ 0.08

TABLE 3. Mean ($\pm$ SE) oxygen uptake rates ($\mu\text{L O}_2 \cdot \text{mg dwt}^{-1} \cdot \text{h}^{-1}$) and aerobic scope of *N. obscura* ($n = 3$), 1, 20, 24, 44, 48, and 68 h after initial placement in the respirometer chambers. ANOVA indicated that no significant differences occurred among the respective time periods in resting or active metabolism or aerobic scope ($n = 200$ oxygen determinations at each time period). Active metabolism is always significantly higher (Student's t -test, $P < 0.05$) than the corresponding resting values.

Time (h)	Resting	Active	Aerobic scope
1	0.51 $\pm$ 0.03	2.13 $\pm$ 0.12	1.62 $\pm$ 0.14
20	0.64 $\pm$ 0.16	2.21 $\pm$ 0.20	1.57 $\pm$ 0.17
24	0.64 $\pm$ 0.12	2.12 $\pm$ 0.10	1.47 $\pm$ 0.06
44	0.69 $\pm$ 0.18	2.12 $\pm$ 0.11	1.50 $\pm$ 0.17
48	0.68 $\pm$ 0.09	2.03 $\pm$ 0.11	1.35 $\pm$ 0.19
68	0.67 $\pm$ 0.08	2.14 $\pm$ 0.23	1.45 $\pm$ 0.29

Experiment IV

Experiment IV assessed intra- and interspecific differences in metabolism between two size classes of summer *N. obscura* and *E. montezuma* leeches and the amphipod *H. montezuma*. The resting and active metabolic rates and corresponding aerobic scope of three individuals of each size class ($n = 1000$ replicate readings for each individual) were measured for 3 h under similar experimental conditions. Student's t -test was used to test for size-related intra- and interspecific differences in metabolic rates.

Results

Significant (ANOVA, $P < 0.05$) among-individual differences were found in the resting and active metabolism and aerobic scope of *N. obscura* of similar weight (Table 1). In addition, the activity patterns differentiating resting and active movements were clearly distinguishable (see Wrona and Davies 1984).

Table 2 shows the changes in oxygen in the control chamber and experimental chamber 1 after 24, 48, and 72 h, in experimental chamber 2 after 48 and 72 h, and in experimental chamber 3 after 72 h. In all cases, no significant differences (Student's t -test, $P > 0.05$) were found between experimental chambers compared with their respective controls, indicating that overall oxygen uptake by bacterial accumulation over the experimental period was undetectable.

No significant differences (ANOVA, $P > 0.05$) were observed in mean resting and active metabolic rates and the mean aerobic scope of three *N. obscura* over a 72-h period (Table 3). However, the respiration rates of active animals were significantly higher (Student's t -test, $P < 0.05$) than for resting animals (Table 3).

The oxygen uptake rates of resting winter and summer *N. obscura*, *E. montezuma*, and *H. montezuma* displayed similar declining trends with increasing body size (Tables 1, 3, and 4). The respiration rates of active individuals of all size classes

were significantly higher (Student's t -test, $P < 0.05$) than obtained for resting individuals, although no significant size-related differences in aerobic scope were found either within winter and summer *N. obscura* or amongst the other species. When similar-sized animals (160 mg wwt) were considered, although the oxygen uptake rate of summer *N. obscura* was lower, the aerobic scope was significantly higher (Student's t -test, $P < 0.05$) than for *E. montezuma*, even though the activity patterns of *E. montezuma* were identical to those exhibited by *N. obscura*. The activity patterns for resting and active *H. montezuma* could be discriminated (Fig. 4).

Discussion

Although several flow-through respirometer systems have been proposed (Gnaiger 1983a, 1983b; Lampert 1984, 1986; Wrona and Davies 1984), the system outlined in this paper incorporates several major advancements. First, because of the incorporation of an activity monitoring system, it is possible to readily determine standard, routine, and active metabolism, activity-specific metabolic costs, and diel patterns in activity and/or respiration. Short-term changes in respiration associated with functions such as digestion and assimilation (i.e. specific dynamic action) can also be assessed, and any activity-related biases (e.g. Carefoot 1990a, 1990b, 1990c) can be easily corrected for. Second, computer automation allows switching between the chambers and permits long-term studies necessary to measure chronic metabolic responses to toxicants.

The results from the four experiments demonstrate the versatility and capability of the respirometer system to produce repeatable and reliable results in both short-term and long-term monitoring of metabolism.

Experiment I illustrates the very high precision of the system, and it was possible to discriminate among-individual differences in resting and active metabolism of *N. obscura* of very similar body size.

Experiment II (Table 2) verifies that the flow-through design minimizes the potential bias from bacterial/mucous accumulation within the respirometer chambers during long-term (72 h) monitoring of metabolism. Thus, reliable estimates of metabolism are possible for long periods even for soft-bodied invertebrates such as freshwater annelids which often produce copious amounts of mucus and/or other secretions that accumulate in closed-bottle systems. Glazier (1991) justified the use of a closed-bottle system for assessing the metabolism of the cladoceran *Daphnia magna* based on the findings of Lampert (1986) indicating that a flow-through and closed-bottle system produced comparable estimates. Although this may hold for short-term studies of metabolism (e.g. 6 h) for certain taxa, respiration in closed-bottle systems is being measured under progressively decreasing oxygen concentration and accumulating waste products. In our system, we adjust the flow rate and chamber size to maintain approximately a 5-torr difference between the experimental animal and the control chamber. This

TABLE 4. Mean ($\pm$ SE) resting and active metabolic rates and aerobic scope ($\mu\text{L O}_2 \cdot \text{mg dwt}^{-1} \cdot \text{h}^{-1}$) of two size groups of the leeches *N. obscura* and *E. montezuma* and two size groups (length) of the amphipod *H. montezuma* ($n = 3$ individuals for each size group with 1000 oxygen determinations for each individual). Values with the same superscript are significantly different at the $P < 0.05$ level (Student's t -test).

Species	wwt (mg) or length (mm)		Resting	Active	Aerobic scope
		dwt (mg)			
<i>N. obscura</i>	26 mg	4.5	1.62 ± 0.24^a	5.16 ± 0.49^d	3.54 ± 0.52
	160 mg	30.0	0.84 ± 0.16^a	3.31 ± 0.29^d	4.15 ± 0.25
<i>E. montezuma</i>	160 mg	22	3.23 ± 0.80^b	6.21 ± 0.35^e	2.79 ± 0.49
	230 mg	28	1.51 ± 0.22^b	3.85 ± 0.54^e	2.34 ± 0.42
<i>H. montezuma</i>	3 mm	0.58	4.02 ± 0.65^c	9.95 ± 1.90^f	5.93 ± 1.25
	6 mm	1.40	1.82 ± 0.21^c	5.69 ± 0.92^f	3.87 ± 0.74

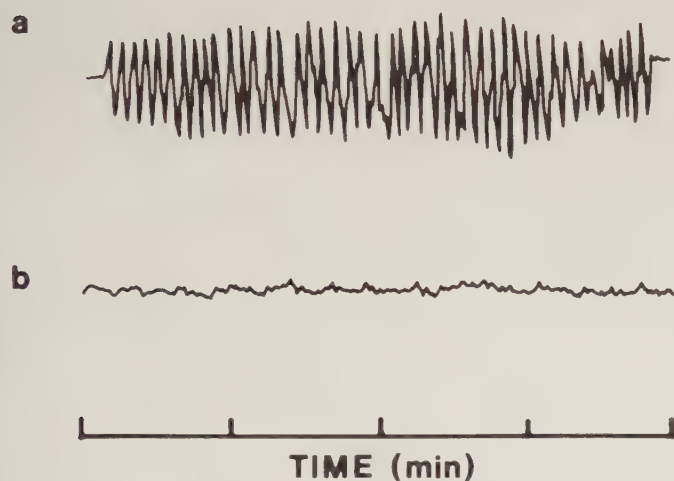


FIG. 4. Impedance activity patterns shown by *H. montezuma* when (a) swimming and (b) at rest.

maximizes the turnover rate of the water within a respirometer chamber, thereby minimizing any stress induced by hypoxia and/or waste accumulation.

Acclimation time to the experimental conditions in the respirometer is also critical to obtain meaningful measurements (Kamler 1969; McMahon et al. 1974). The main advantage of using a flow-through system is that constant abiotic environmental conditions can be established and maintained for a sufficient time to allow acclimation (Kamler 1969). By appropriately adjusting the chamber volume and the flow rate relative to the size of the animal, the acclimation time can be substantially reduced. Experiment III shows that *N. obscura* acclimates quickly (<1 h) to experimental conditions, as no significant difference was observed in mean resting and active metabolic rates over the 72-h experimental period, although active metabolism was consistently higher than resting (Table 3).

Finally, experiment IV shows the flexibility of the system in determining size-dependent intra- and interspecific differences in metabolism. Significant differences were observed in different size groups of summer *N. obscura*, *E. montezuma*, and *H. montezuma* and between *N. obscura* and *E. montezuma* of the same size. Similarly, experiments I and III show size-related differences in winter *N. obscura*. Hence, the system is very adaptable for comparative studies of metabolism.

Another important feature of the respirometer system described here is the ability to monitor activity patterns and oxygen uptake simultaneously. With other closed and flow-

through respirometers, assumptions are often made regarding the type of metabolism being measured (i.e. resting, routine, or active) (e.g. Berg and Jonasson 1965; Calow 1975; Sweeney and Vannote 1981; Glazier 1991), or direct observations of activity are made on uncovered chambers which are then correlated with the measured levels of oxygen uptake (e.g. Knight and Gauvin 1966; Nagell 1973). In addition, many organisms show diel cycling of metabolic rates (Ultsch et al. 1980) which although not present in *N. obscura*, *E. montezuma*, or *H. azteca* could be easily discerned using our system.

Gnaiger (1983b) and Lampert (1986) have proposed twin flow-through respirometer designs; however, they incorporate only a single experimental animal chamber, do not monitor activity, or are not automated to the same extent as our system. By utilizing Labtech Notebook software and the ADALAB data acquisition system, our system allows for a great deal of flexibility in determining the duration and sampling frequency of oxygen tension and activity. Also, since all data (time, temperature, chamber number, oxygen tension, and activity output) are stored directly onto a hard disk for future analysis, this immediately frees the system for experimental runs. The modular design of the system makes it possible to adapt it easily and quickly for studying the metabolism of a wide variety of aquatic organisms exposed to an array of ecological circumstances (e.g. acute and chronic hypoxia and/or toxicant levels, varying food regimes, etc.).

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Seasonal Variation in the Physiological Response of Atlantic Cod (*Gadus morhua*) to Low Salinity

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Plasma Na^+ , Cl^- , K^+ , osmotic pressure, cortisol, glucose, and protein, blood hemoglobin and hematocrit, and water content of skeletal muscle were measured at regular intervals during a 28-d period following the transfer of Atlantic cod (*Gadus morhua*) to waters of 7, 14, 21, and 28‰ (control) salinity. These experiments were repeated four times at 3-mo intervals under natural photoperiod and temperatures (0–10°C). Exposure to 7‰ salinity caused large decreases in plasma Na^+ in winter (25 mmol/L over 14 d) and in spring (32 mmol/L over 7 d) when the lowest value for the year was reached (156 mmol/L). Transfer to 14 and 21‰ salinity resulted in a slight decrease (maximum 4%) in plasma Na^+ which was much smaller than the seasonal variation (14%) observed in controls. Hydration of skeletal muscle occurred only at 7‰ (2.3% maximum), but these changes were small compared with the seasonal variation (3.9%) observed in the controls. Principal components and clustering analyses showed that all ionic and osmotic variables measured were highly correlated while being only weakly associated with the condition or reproductive status of the fish. There were no indications that acclimation to low salinity was stressful for cod.

Nous avons mesuré, à intervalles réguliers, chez des morues franches (*Gadus morhua*) exposées pendant 28 d à des salinités de 7, 14, 21 et 28‰ (témoin) les concentrations de Na^+ , Cl^- , K^+ , pression osmotique, cortisol, glucose et protéines plasmatiques, l'hémoglobine, l'hématocrite et le contenu en eau du muscle. Les morues ont été soumises aux variations naturelles de photopériode et de température (0–10°C) et quatre expériences ont été réalisées à 3 mo d'intervalle l'une de l'autre. Le Na^+ plasmatique a chuté en hiver (25 mmol/L en 14 d) et au printemps (32 mmol/L en 7 d) à 7‰, alors que la concentration a atteint une valeur minimale annuelle de 156 mmol/L. À 14 et 21‰, la baisse maximale du Na^+ fut de seulement 4 %, soit une valeur moindre que l'écart saisonnier de 14 % chez les témoins. L'hydratation du muscle, observée uniquement à 7‰, n'a jamais été de plus de 2.3 %, alors que les témoins montraient un écart saisonnier de 3.9 %. Les analyses multivariées ont démontré que les variables ioniques et osmotiques étaient fortement corrélées entre elles et faiblement associées à l'état nutritionnel ou reproducteur des individus. Rien n'indique que l'acclimation à ces faibles salinités entraîne un stress chez la morue.

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Most studies on the ability of fish to tolerate changes in salinity have been done on salmonids transferred from fresh to seawater (see reviews by Folmar and Dickhoff 1980; McCormick and Saunders 1987; Hoar 1988; Virtanen 1988). Marine fishes in general do not survive exposure to freshwater. However, some marine species have been shown to tolerate very low salinities and even freshwater (Woo and Fung 1981; Woo and Wu 1982; Wu and Woo 1983; Vislie and Fugelli 1984).

Atlantic cod (*Gadus morhua*) can tolerate prolonged exposure to low salinities as their presence in the Baltic suggests; however, our knowledge of their ability to survive in euryhaline conditions is limited to a few studies conducted at different stages in their life history. Percentages of fertilized and normally developed cod eggs decreased at salinities below 10–12.5‰ (Davenport et al. 1981; Westin and Nissling 1991). Low salinity delayed hatching (Laurence and Rogers 1976), but

cod larvae have been shown to develop normally at 22‰ (Dannevig and Silvertsen 1933). Fletcher (1978a, 1978b) quantified ionic and osmotic regulation in 250- to 1000-g cod over periods of up to 10 h at salinities ranging from fresh to 52‰ seawater. The mortality rate of 1.8-kg cod was monitored while gradually decreasing salinity over 120 d (Odense et al. 1966); behavioral changes were observed at salinities below 5‰, with mortality occurring below 3‰. Harden Jones and Scholes (1974) studied the effect of temperature on plasma Cl^- concentrations in 30- to 45-cm cod. Some of their experiments carried out at low salinity led them to conclude that warm (10°C), low-salinity (7–8‰) water may be lethal for cod.

Because cod may eventually be used for aquaculture in coastal regions with brackish water, we investigated seasonal changes in the ability of cod to tolerate direct exposure to salinities below 30‰. Osmoregulatory and ionoregulatory capabilities were assessed by measuring Na^+ , Cl^- , K^+ , osmotic

pressure, and total protein concentrations in plasma and the water content of skeletal muscle. To determine whether or not the osmotic changes resulted in a high level of stress, our experiments were designed to separate these stress responses from those that might be caused by manipulation or sampling conditions. To evaluate the level of stress response, we measured blood hemoglobin and hematocrit and plasma glucose and cortisol, i.e. physiological parameters likely to be altered by exposure to a stressor in teleost fish (Donaldson 1981; Soivio and Nikinmaa 1981; Schreck 1990; Thomas 1990). Changes in these parameters in response to stress have been measured in marine species by Wardle (1972), Ishioka (1980), White and Fletcher (1986), and Kacem et al. (1986, 1987).

Material and Methods

Experimental Procedures

Cod were caught by trawl in the Gulf of St. Lawrence near Matane (Québec) in October 1988 and June 1989. They were kept under natural photoperiod, salinity, and temperature conditions for 1–6 mo before being used for experiments. Fish were held in 13-m³ tanks at densities that never exceeded 28 kg/m³. Flow rates averaged 50 L/min and dissolved oxygen was maintained above 75% saturation. Fluorescent lights were controlled by automatic timers adjusted weekly to follow the natural photoperiod. Seawater was pumped from a depth of 15 m into reservoirs and was distributed to rearing tanks at temperatures ranging from 0°C in January to 10°C in late summer. Salinity averaged 28‰ (range 24–30‰) over the 12-mo experimental period. Fish were fed a maintenance ration twice weekly alternately with smelt or capelin and with a moist feed that was a mixture of marine fish, invertebrates, and vitamins.

Survival experiments were done by transferring cod directly to five 1.5-m³ tanks at 0, 7, 14, 21, or 28‰ salinity (flow rate 20 L/min). Mortality rates were monitored over 96 h using video cameras. Twenty cod were transferred into each experimental tank except for the freshwater tank which received 10 cod. The experiment was repeated four times in a 1-yr period (November 1988 and January, March, and July 1989). Fish were not fed during these experiments.

Physiological experiments consisting of direct transfers of 90 fish to each of four 8-m³ tanks at 7, 14, 21, or 28‰ (flow rate 20 L/min) were started 1 wk after each survival experiment. Ten cod were sampled from each tank after 1, 2, 3, 4, 7, 14, 21, and 28 d, except in the fall experiment at 28‰ when no sampling was done on days 2, 3, 4, and 21. Ten cod were also sampled from the holding tanks on days 1 and 28 during the spring and summer experiments. Feeding was stopped 48 h before the experiments. Fish were fed a maintenance ration once or twice weekly (depending on temperature) from the second week of the experiments, but they were starved at least 48 h before sampling.

Diluted seawater was obtained by mixing unchlorinated aerated well water (0.3–0.4‰ salinity) and seawater using manually controlled flowmeters. Salinity was measured and adjusted daily. Seasonal photoperiod and temperature were similar between experimental and holding tanks. However, temperature was slightly warmer in tanks receiving diluted seawater (<1°C) or freshwater (1°C) than in tanks at 28‰.

Fish were stunned by a blow to the head and blood was drawn from the caudal vein with an ammonium-heparinized syringe within 5 min. Blood aliquots were used for hematocrit and hemoglobin measurements. Plasma was separated by

centrifugation, divided into aliquots, and frozen at –80°C until further analysis. Each cod was measured (total length (TL) $\pm$ 1 mm) and weighed ($\pm$ 1 g). Sex was determined and gonads were weighed ($\pm$ 1 mg). The water content of skeletal muscle from the dorsolateral area behind the head was determined by drying a 10-g sample to constant weight at 80°C.

Plasma Na⁺, Cl[–], and K⁺ were measured with a multiple-ion analyser using ion-selective electrodes (Ciba Corning, model 644). A microosmometer (Advanced Instrument, model 3 MO) was used for osmotic pressure determination. The colorimetric method of Bradford (1976) using bovine albumin as standard was used to measure plasma proteins. Blood hematocrit was read following centrifugation at 10 000 rpm for 5 min. Blood hemoglobin was measured by the cyanmethemoglobin method (Sigma, No. 525 A). Plasma glucose was determined by an enzymatic assay (Sigma, No. 16 UV) and plasma cortisol by radioimmunoassay (Kallestad, No. 825). Because cortisol levels in cod plasma were much lower than in human plasma for which the kit had been developed, we extended the standard curve down by diluting the lower standards using the cortisol-free plasma (standard 0) provided with the kit. Hematocrit, hemoglobin, glucose, and cortisol were not measured on days 2, 3, and 4. All measurements were done in duplicate except for the water content of muscle. Mean cell hemoglobin content (MCH) was calculated as the ratio of hemoglobin to hematocrit. Gonadosomatic index (GSI) and condition factor (CF) were calculated as follows:

$$\text{GSI} = 100 \cdot \text{GW} / (\text{PW} - \text{GW})$$

$$\text{CF} = \text{MW} / \text{PW}$$

where GW is gonad weight (grams), MW is measured fish weight (grams), and PW is fish weight (grams) as predicted from the weight–length relationship determined for all the cod used in the experiments. Mean length and weight of cod were 417 $\pm$ 2.8 mm and 596 $\pm$ 12.9 g (fall), 411 $\pm$ 2.5 mm and 539 $\pm$ 10.5 g (winter), 417 $\pm$ 2.4 mm and 552 $\pm$ 11.1 g (spring), and 488 $\pm$ 1.9 mm and 718 $\pm$ 9.5 g (summer).

Statistical Analysis

The large number of variables considered led us to begin the statistical analysis with a reductive approach. Principal components analysis was used to show graphically relations between the variables within the first two principal axes of variation of the data. Clustering procedure analysis aimed at grouping variables using their correlation through all axes of variation. Detailed objectives were to (1) identify sets of highly correlated variables, (2) extract a single representative variable from each set, (3) select among the representative variables the one showing the greatest variation, the latter being further on considered as the main response to the salinity treatment, and (4) examine through angular proximity and common cluster the relations between the representative variables and the main response to the salinity treatment.

Principal components analysis and cluster analysis (Legendre and Legendre 1984) were performed using the FACTOR-PRINCIPAL and CLUSTER-AVERAGE procedures in SAS (SAS Institute 1985). Na⁺, Cl[–], osmotic pressure, hemoglobin, hematocrit, MCH, and protein data were first inverted by subtracting x_i from the maximum value ($(\max x) + 1$) to ensure positive skewness for all variables. The square root transformation was then applied to all variables except the cortisol data which were log-transformed. Principal components analysis was performed on the correlation matrix to homogenize the dif-

ferent scales of measurement of the variables; eigenvectors were standardized using the square root of the eigenvalue in order to represent graphically correlations between variables and principal axes (Legendre and Legendre 1984). For the clustering procedure, the matrix of distances was formed by using the complement of the absolute value of the correlation coefficient. The average linkage method was selected because of the systematic nature of our sampling schedule (Legendre and Legendre 1984).

The second step of the analysis consisted of describing the physiological responses using standard tests of analysis of variance, analysis of covariance, and multiple comparisons. Measured physiological parameters were treated as dependent variables; independent variables included classification factors (salinity, season, day of sampling) and continuous covariables (GSI, CF). Following the same transformation criteria as for multivariate analyses, no transformation was applied to data on muscle water, plasma Na^+ , protein, and blood hemoglobin; data on plasma glucose, cortisol, length, and weight were log-transformed; CF data were transformed to square root values. Probability levels were based on partial sums of squares (type III) which assess the independent contribution of each factor being tested (Freund and Littell 1981). When the analyses of variance were significant, multiple comparisons were made using the Tukey–Kramer method (SAS Institute 1985). Tukey–Kramer tests were done for each level of a classification factor when an interaction term was significant. Statistical analyses were all done using various utilities within the GLM procedure of SAS software (SAS Institute 1985).

Results

Survival Experiments

Survival rate was 100% following direct transfer to any salinity above 0‰, but no cod survived direct transfer to freshwater. Time to 50% mortality in freshwater never exceeded 8 h in any season. No mortality occurred among cod held at 7, 14, or 21‰ salinity for longer periods (up to 28 d) in the physiological response experiments.

Multivariate Analyses

The results from the principal components analysis on the correlation matrix of the variables are presented in Fig. 1. The angular proximity to the principal axes and the length of the vectors indicate that factor I is mostly contributed by plasma Na^+ , Cl^- , osmotic pressure, protein, muscle water, and CF. Because of the preeminent position of the ionic and osmotic variables, this axis most likely comprises the variables correlated with the experimental conditions. Conversely, length, weight, hemoglobin, and hematocrit, the main contributors to factor II, represent variables mostly independent of the ionic and osmotic responses.

Clustering proceeded through a span of average distances from 0.2 to 1.1. Primary groups of variables (Fig. 1) were created at a distance of 0.4, thus linking strongly associated variables. Primary group 1 included plasma Na^+ , Cl^- , and osmolality; primary group 2, muscle water, plasma protein, and CF; primary group 3, length and weight; and primary group 4, blood hemoglobin and MCH. The clustering of primary groups into secondary groups (Fig. 1) occurred only at a distance of 1.0, indicating a moderate or weak link between the primary groups. Secondary group A included primary groups 1 and 2 plus GSI and plasma K^+ ; secondary group B, primary groups 3

and 4 plus hematocrit; and secondary group C, plasma cortisol and plasma glucose.

Because plasma Na^+ , Cl^- , and osmolality were strongly associated variables, an analysis of Na^+ only, which showed the largest variation and strongest association with factor I, was considered representative of any of the three. Although muscle water and plasma protein levels were closely associated, both were retained for subsequent analyses because they represented different physiological compartments. CF and GSI were at least loosely associated with the above two primary groups; they were kept for use as covariables. Cortisol and glucose were only weakly associated with factor I. However, in view of their potential as indicators of stress, this connection was explored in a separate analysis.

Plasma Na^+

The response of cod to decreased salinity was moderate at the intermediate salinities tested (14 and 21‰) but was more pronounced at 7‰. The response also varied from season to season (Fig. 2). In winter and spring, plasma Na^+ decreased during the first 4 d following exposure to experimental salinities ($P < 0.001$). This decrease was particularly pronounced at 7‰. During the second week, plasma Na^+ remained low in winter whereas a partial recovery was observed in spring (Fig. 2b and 2c). In fall and summer, no such decreases were observed during the first 4 d of the experiments ($P \geq 0.24$). However, in the second week, slight differences were observed ($P \leq 0.003$; Fig. 2a and 2d). By the end of the experiments (days 21 and 28), new steady-state conditions were established. The final mean concentrations decreased with decreasing water salinity ($P < 0.001$; Table 1). Plasma Na^+ was generally highest in the controls (28‰) and lowest at 7‰. There were no significant differences between the two intermediate salinities (14 and 21‰). Plasma Na^+ in cod exposed to 7‰ failed to return to the values observed at higher salinities, particularly in winter and spring ($P < 0.05$; Fig. 2b and 2c). Table 1 also shows that plasma Na^+ values were similar in transferred and untransferred control animals, suggesting no transfer effect.

Interestingly, a marginally significant negative correlation of plasma Na^+ with CF was observed in all seasons ($P < 0.05$) except spring ($P = 0.31$). Conversely, plasma Na^+ was negatively correlated with GSI only in spring ($P < 0.01$), i.e. during the late maturation period. CF and GSI contributed, however, to a very low proportion of the explained variance in our model ($<4\%$ in any season) and thus can be considered as having a negligible influence overall. Within-season variability in plasma Na^+ was mainly associated with changes in salinity ($P < 0.001$; 48–81% of the explained variance, EV). There were also differences between days ($P < 0.002$; 9–36% EV), and in winter, spring, and summer, both factors interacted ($P < 0.003$; 8–28% EV).

Control animals: seasonal differences

Seasonal differences observed between controls were largely attributable to water temperature. In cod transferred to control conditions, season accounted for most of the variability in plasma Na^+ ($P < 0.001$; 91% EV). In contrast, between-day differences and interaction between day and season were negligible ($P < 0.001$; 2 and 7% EV, respectively). The small percentage of variation explained by day of sampling suggests that short-term variations in water temperature, such as those observed during the fall and spring experiments, and handling cod for transfer had little influence on Na^+ levels. When results for the last week of the experiments (days 21 and 28) were

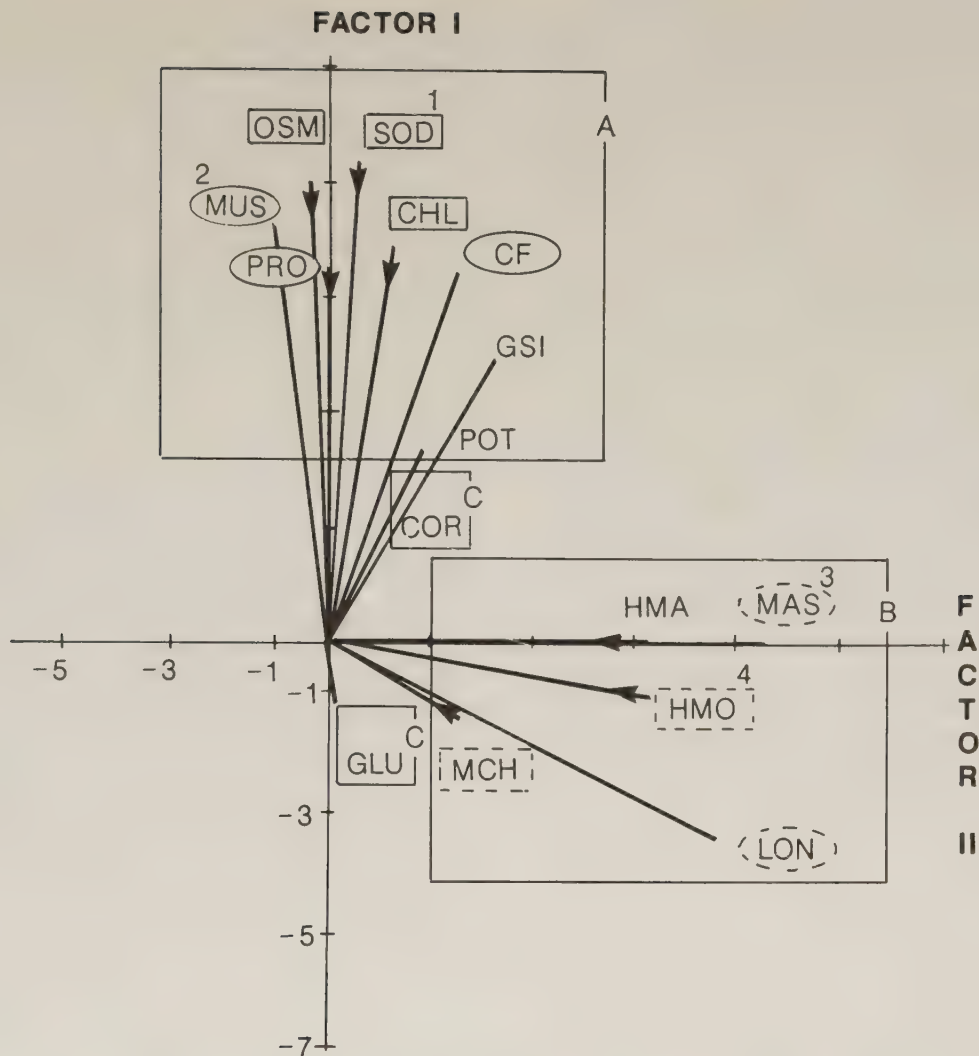


FIG. 1. Position of the vector-variables in the plan of the first two principal components and results of the clustering procedure. Variables marked with common symbols comprise a cluster: numbers identify primary clusters (closely associated variables); letters indicate secondary clusters (loosely associated variables). SOD, plasma Na^+ ; CHL, plasma Cl^- ; POT, plasma K^+ ; OSM, plasma osmotic pressure; MUS, muscle water content; PRO, plasma total protein; CF, condition factor; GSI, gonadosomatic index; HMA, blood hematocrit; HMO, blood hemoglobin; MCH, mean cellular hemoglobin content; LON, fish length; MAS, fish weight; COR, plasma cortisol; GLU, plasma glucose. Arrows indicate positive direction of inverted variables.

compared, seasonal differences were still present ($P < 0.001$), with the lowest Na^+ levels being observed in summer (167.9 ± 0.9 mmol/L (mean $\pm$ SE); $P < 0.05$) and the highest in winter (195.5 ± 0.5 mmol/L; $P < 0.05$). No difference was observed between the fall and spring plasma Na^+ levels (182.7 ± 1.7 and 181.3 ± 0.6 mmol/L; $P > 0.05$). Differences in plasma Na^+ followed differences in temperature conditions. Temperature averaged $1.2 \pm 0.1^\circ\text{C}$ in winter and $9.4 \pm 0.2^\circ\text{C}$ in summer; conditions were similar in fall ($4.4 \pm 0.2^\circ\text{C}$ decreasing) as in spring ($4.7 \pm 0.1^\circ\text{C}$ increasing). No relation was found between plasma Na^+ and CF or GSI ($P > 0.23$).

Transfer to 7‰ salinity: seasonal differences

Differences in plasma Na^+ concentrations between seasons at 7‰ were significant ($P < 0.001$; 42% EV), but the day of sampling and the interaction between both factors had a greater influence at 7‰ ($P < 0.001$; 24 and 27% EV, respectively) than at 28‰ salinity. During the last week of the experiments

(days 21 and 28), summer values (162.7 ± 0.7 mmol/L) differed significantly ($P < 0.05$) from those observed in fall (172.7 ± 0.8 mmol/L), winter (177.3 ± 2.3 mmol/L), and spring (172.3 ± 1.7 mmol/L) (Fig. 2), representing a smaller overall variation in Na^+ , however, than at 28‰. The lowest Na^+ concentrations were observed on days 3–7 during the spring experiment at 7‰ salinity. Figure 3 compares the lowest values observed at 7‰ (spring experiment) with the lowest values observed in the controls (summer experiment). Differences in plasma Na^+ between 7‰ in spring and 28‰ in summer were significant ($P < 0.001$) as were those between days ($P < 0.001$). The interaction was also significant as suggested in Fig. 3 ($P < 0.001$). By days 21 and 28, however, plasma Na^+ had increased at 7‰ salinity in the spring experiment to a level similar to that in controls in the summer experiment ($P = 0.44$). Although the relation was significant ($P < 0.001$), the response of plasma Na^+ to 7‰ salinity was not really affected by the processes of sexual maturation (5% EV) or CF ($P = 0.056$; 1% EV).

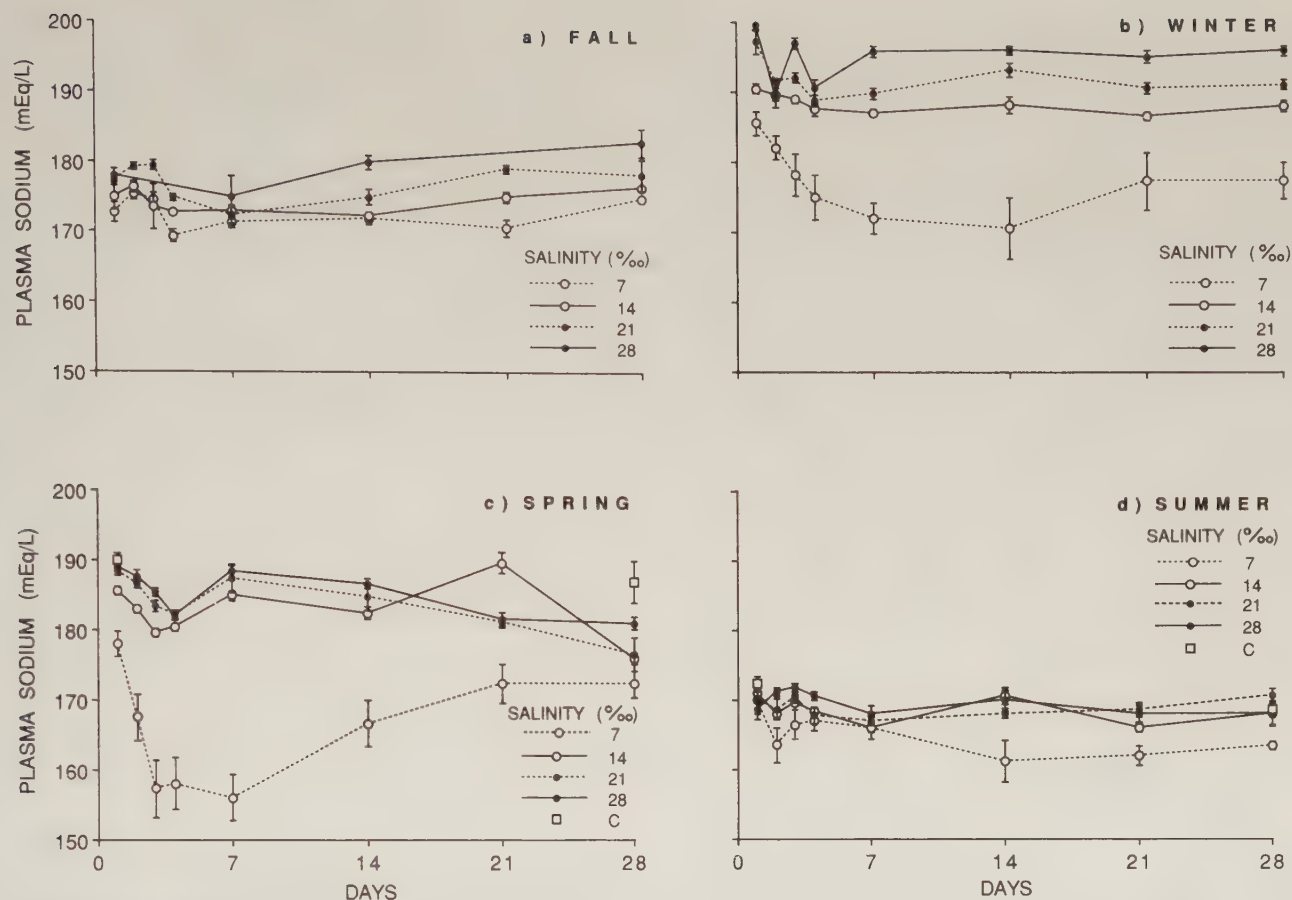


FIG. 2. Time course changes in plasma sodium concentration in cod directly transferred from 28‰ to 7, 14, 21, and 28‰ salinity. Each point represents the mean value $\pm$ SE ($n = 10$). Temperature averaged (a) $4.4 \pm 0.2^\circ\text{C}$ over the 28-d period in fall, (b) $1.2 \pm 0.1^\circ\text{C}$ in winter, (c) $4.7 \pm 0.1^\circ\text{C}$ in spring, and (d) $9.4 \pm 0.2^\circ\text{C}$ in summer. The value for untransferred controls is also shown for days 1 and 28 (Fig. 2c and 2d).

TABLE 1. Mean and SE of plasma Na^+ concentrations of cod directly transferred to three experimental salinities (7, 14, and 21‰) and one control salinity (28‰) after 21 and 28 d of exposure. Values for untransferred animals are also shown (U). Days 21 and 28 are presented separately when significant differences were observed. Multiple means comparisons are based on the Tukey-Kramer's test; means sharing the same superscript in a row are not significantly different ($P \geq 0.05$).

Season	Day	Sodium concentration (mmol/L)				
Fall	21-28	28 ^a	21 ^b	14 ^{b,c}	7 ^c	
		182.7 ± 1.7	178.7 ± 1.1	175.8 ± 0.5	172.7 ± 0.8	
Winter	21-28	28 ^a	21 ^b	14 ^b	7 ^c	
		195.4 ± 0.5	190.8 ± 0.5	187.3 ± 0.5	177.3 ± 2.3	
Spring	21	14 ^a	28 ^b	21 ^b	7 ^c	
		189.7 ± 1.4	181.5 ± 1.0	181.2 ± 0.7	172.4 ± 2.8	
	28	U ^a	28 ^{a,b}	21 ^{b,c}	14 ^{b,c}	7 ^c
		187.3 ± 3.2	181.1 ± 0.8	176.6 ± 2.5	176.0 ± 0.8	172.3 ± 1.9
Summer	21-28	21 ^a	U ^a	28 ^a	14 ^a	7 ^b
		169.6 ± 0.7	168.6 ± 0.8	167.9 ± 0.9	166.5 ± 0.9	162.7 ± 0.7

Plasma Total Protein and Water Content of Muscle

The concentration of protein in the plasma and the water content of muscle changed very little in response to various salinities. Water content of muscle increased in cod exposed to 7‰ salinity in winter and spring, when the plasma Na^+ response was pronounced. For the first 4 d of the experiments, the water content of muscle increased by 1.3% (winter experiment; $P < 0.05$) and 2.3% (spring experiment; $P > 0.05$) at

7‰ salinity. This was reduced to 1.0% ($P < 0.05$) and 1.4% ($P < 0.05$) in the second week and to 0.8% ($P < 0.05$) and 0.5% ($P > 0.05$) by the end of the experiments. For plasma protein, no consistent pattern emerged.

The measured changes in muscle water and plasma protein levels in response to low salinity represent only small differences from control levels; season and CF had a much greater influence than salinity. Season accounted for as much as 90%

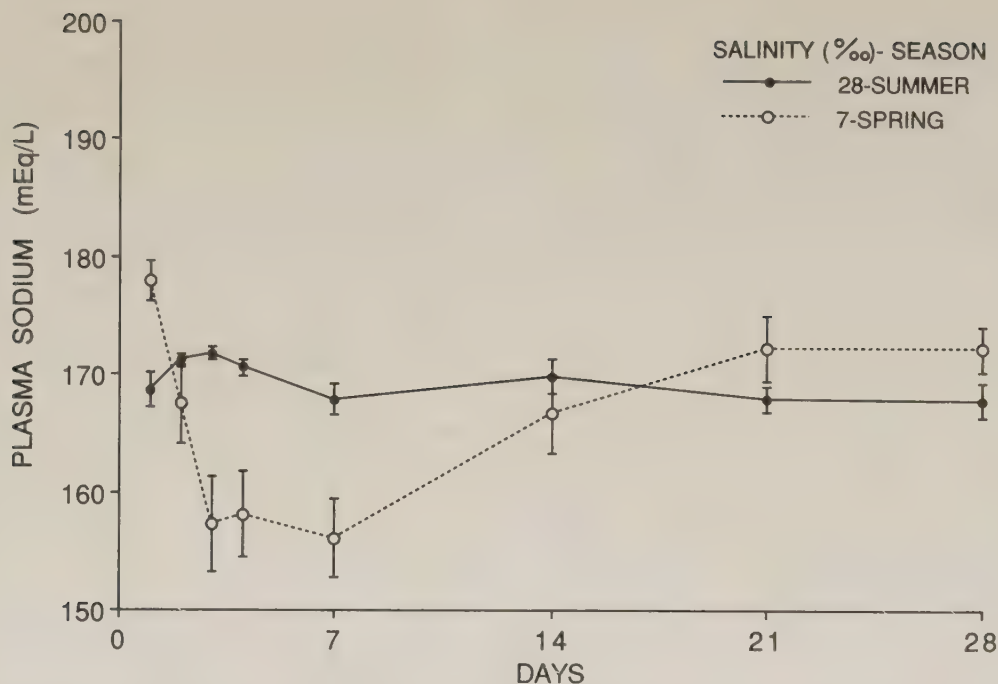


FIG. 3. Time course changes in plasma sodium concentration in cod transferred directly from 28 to 7‰ salinity in spring and from 28 to 28‰ salinity in summer. Each point represents the mean value $\pm$ SE ($n = 10$).

of the observed variability in muscle water (80.5% in fall to 84.4% in summer) and plasma protein (30.0 g/L in fall to 17.2 g/L in summer) levels in control conditions ($P < 0.001$). CF accounted for more than 50% of the variability in muscle water and plasma protein levels in control and experimental animals when seasons were considered individually. In contrast, GSI accounted for less than 5% of that variability.

Hemoglobin, Hematocrit, Cortisol, and Glucose

Exploratory analyses on the main data set failed to demonstrate any relation between salinity and the following parameters: hemoglobin, hematocrit, cortisol, and glucose. Multivariate analyses, on the other hand, indicated a weak link among plasma cortisol, plasma glucose, and the osmoionic response (Fig. 1). Cortisol increased and glucose decreased with decreasing plasma Na^+ . In order to better characterize the magnitude of this relation, we focused on comparisons between two extreme situations found in our experimental design.

Cortisol and glucose data were analyzed in cod transferred to 7‰ salinity during the spring (days 1–28), i.e. when the most pronounced plasma Na^+ response was observed. These parameters were also analyzed in cod transferred to the control salinity (28‰) and sampled on days 21 and 28. For control cod, seasons were pooled to obtain a range in Na^+ concentrations similar to that obtained at 7‰ during the spring. In the two analyses the rank of sampling was also taken into account to determine if this aspect of the experimental procedure was an important factor. Only significant results are reported below.

In spring, in cod exposed to 7‰ salinity, both cortisol and glucose covaried with plasma Na^+ ($P < 0.01$); however, the osmotic status of the fish only explained a small proportion of the variability (12 and 14% for cortisol and glucose, respectively). Below 160 mmol Na^+ /L, cortisol increased to a mean value of 4.0 $\mu\text{g}/100 \text{ mL}$ and a maximal observed concentration of 11 $\mu\text{g}/100 \text{ mL}$ (regression slope -0.019 , intercept -3.94 ,

$P < 0.01$, $r^2 = 0.06$). In comparison, cortisol concentration averaged $2.0 \pm 0.4 \mu\text{g}/100 \text{ mL}$ for all treatments in spring ($n = 256$). The reverse tendency was observed for plasma glucose (regression slope 0.006, intercept 3.05, $P < 0.001$, $r^2 = 0.06$). Cod having plasma Na^+ concentrations lower than 160 mmol/L averaged 43 mg/100 mL and never exceeded 65 mg/100 mL of plasma glucose. This is below the mean concentration for all treatments in the spring experiment ($60.1 \pm 1.1 \text{ mg}/100 \text{ mL}$; $n = 256$). The duration of exposure to 7‰ and the rank of sampling had no significant effect on plasma cortisol, although these two factors had small but significant effects on plasma glucose ($P < 0.05$). Plasma glucose decreased with duration of exposure and increased with rank of sampling.

In control cod, which exhibited a wide seasonal range in plasma Na^+ , cortisol was correlated with plasma Na^+ and rank of sampling (regression slope -0.023 and 0.081 , respectively, intercept 4.57, $P < 0.05$). The highest cortisol concentrations were observed in the last three fish sampled from the tanks (10 fish per experimental condition per day), but only a few fish displayed such a response. No clear pattern emerged in terms of plasma Na^+ . Each factor accounted for only 7% of the variability in plasma cortisol. No relationship was found between plasma Na^+ or rank of sampling and plasma glucose.

Discussion

Survival experiments indicate that cod are very tolerant of low salinities, but they cannot tolerate freshwater. The lower critical salinity level was found to be below 7‰. Odense et al. (1966) showed that cod of similar size became excited when the salinity (as sodium chloride) reached 4.6‰ and died when the salinity was further reduced to below 3‰. In that study, fish were gradually acclimated to low salinity over a period of 100 d (5–6°C). This would indicate that a period of acclimation does not significantly improve the ability of cod to tolerate low

salinities. Wu and Woo (1983) exposed 20 fish of each of 13 different marine species to low salinities for a period of 2 wk. The fish of 12 species were able to survive at 10‰ salinity; those of six and three species were only able to survive at 5 and 3‰ salinity, respectively.

Seasonal variations in the ability of marine fishes to respond to changing osmotic conditions have not been previously investigated. In this study, no seasonal variability in survival rates of cod exposed to freshwater or to water at 7‰ salinity was observed, but we have shown that there is some degree of seasonal variability in the ability of cod to regulate plasma Na^+ following transfer to 7‰ salinity.

Direct exposure to 14 and 21‰ salinity resulted in only a slight departure from control plasma Na^+ values during any of the four seasons. This was to be expected because the net branchial flux of Na^+ in cod has been shown to be linearly dependent on external salinity down to 12‰ (Fletcher 1978b). By the end of the experiments, discrepancies in plasma Na^+ concentration averaged 6 mmol/L between treatments (14, 21, and 28‰). Seasonal fluctuations among controls were much larger (28 mmol/L), suggesting that perturbations at salinities above 14‰ were negligible compared with normal seasonal fluctuations (see also Fletcher et al. 1982). Similarly, the results obtained with plasma cortisol and plasma glucose confirmed that an exposure to 14 and 21‰ salinity could not be considered as stressful for cod. Like cod, red sea bream (*Chrysophrys major*), a marine species, regulated its body fluids within narrow limits as long as salinity remained above isoosmotic concentrations (160 mmol Na^+ /L, 340 mOsm at 20°C; Woo and Fung 1981). In red grouper (*Epinephelus akaara*) or black sea bream (*Mylio macrocephalus*), the response to decreased salinity was masked by a pronounced stress of transfer (Woo and Wu 1982), but no hypomineralisation of body fluids occurred in red grouper exposed to salinities above 12‰. Similar responses have been observed in other marine teleosts (Oikari 1978). Euryhaline species, i.e. those that survive in both fresh and seawater, generally experience a dilution in body fluids as the external salinity decreases. In the banded killifish (*Fundulus diaphanus*), plasma Na^+ decreased from 185 mmol/L at 30‰ (20°C) to 154 mmol/L at 10‰ (20°C) and to 128 mmol/L in freshwater (Ahokas and Duerr 1975). Dilution of plasma also occurred in threespine stickleback (*Gasterosteus aculeatus*) (Lange and Fugelli 1965) and flounder (*Platichthys flesus*) (Vislie and Fugelli 1984) in freshwater. In contrast, milkfish (*Chanos chanos*), a species that inhabits hypersaline lagoons, maintained plasma ionic and osmotic concentrations within much narrower limits following transfer to salinities ranging from 0 to 32‰ (Ferraris et al. 1988).

Cod in the fall and summer experiments responded similarly to salinities of 7, 14, and 21‰ and did not go through a critical phase of adjustment. However, exposure to 7‰ salinity in winter and spring resulted in a rapid decrease in plasma Na^+ accompanied by a significant increase in muscle water. On the other hand, those changes in plasma Na^+ (32 mmol/L) were of similar amplitude as the seasonal changes observed in the controls (28 mmol/L), and the lowest final Na^+ concentration reached at 7‰ (spring) did not differ from that observed in controls (summer). Nevertheless, because cod exhibited much wider fluctuations in plasma Na^+ in some seasons than in others, when exposed to 7‰, this level of salinity must be near their limit of tolerance. Fletcher (1978b) showed that Na^+ outflux by active transport decreased with external salinity in cod and was inhibited below the isoosmotic concentration. Below 12‰ salinity, cod have to minimize the loss of ions and make

up for ions lost through passive diffusion and urine production. Based on two mortalities out of 10 cod exposed to 7–8‰ salinity (10°C, our summer condition) and on a higher degree of excitability at 10°C than at 0 or 2°C (our winter condition), Harden Jones and Scholes (1974) concluded that warm (10°C), low-salinity (7–8‰) water may be lethal to cod. However, their study was conducted in February, i.e. in winter on cod that were presumably maturing, and they observed the lowest plasma Cl^- concentrations at 0°C rather than at 10°C. Mortalities and excitability might thus be ascribed to water temperature and/or unmeasured factors rather than to low salinity.

No factor can be singled out to explain how the winter and spring experiments may have differed from the summer and fall experiments. CF outside spring and the GSI in spring were correlated with plasma Na^+ , but they explained only a small percentage of the total variability. They were also correlated with plasma Na^+ at 7‰ but not at 28‰ salinity, when seasons were pooled. CF was highest in fall when cod were taken from the wild and it decreased over winter as temperature in our tanks remained near 0°C. Further decreases occurred in spring as the cod matured and the GSI reached high values. The cod used in summer were brought from the wild during the spawning period and their CF remained low until midsummer when the last experiment was conducted.

Plasma protein concentration and water content of muscle also varied seasonally. Plasma protein changes were not correlated with the osmoionic response in the range of salinities that we examined. Water content of muscle increased in cod exposed to 7‰ in winter and spring, but the increase was negligible as compared with the seasonal changes exhibited by controls. Because season accounted for as much as 90% of the explained variability in muscle water and plasma protein levels in controls, these two variables may have fluctuated because CF and GSI varied seasonally. Water content of muscle was found to increase in starving rainbow trout (*Oncorhynchus mykiss*) in both fresh and seawater (MacLeod 1977). Plasma protein concentration declined in fasted red sea bream by as much as 58% in seawater and 30% in isosmotic water over 25 d (Woo and Murat 1981). Season and size also influenced the water content of muscle in wild cod, the maximum being reached in large mature cod (range 79.5–83.0%) (Love 1970). Similar results were found by Damberg (1964) (range 80.6–81.6%). However, Eliassen and Vahl (1982) found similar changes in muscle water content in immature cod during the spawning season (range 81.0–82.2%). Larger increases were seen in the present experiments.

The decrease in plasma Na^+ in cod transferred to 7‰ salinity in the spring occurred concomitantly with an increase in plasma cortisol and a decrease in plasma glucose. The increase in cortisol was small, but Pickering and Pottinger (1989) have shown that an increase as small as 10 ng/mL indicates a chronic stress situation. High cortisol values were observed during the first week of the spring experiment in fish that had the lowest plasma Na^+ concentrations observed in this study. Therefore, the failure to maintain osmotic status above a critical level probably produced the stress effect detected. The decrease in plasma glucose was also of very small amplitude. The results obtained in cod transferred to 28‰ suggest that decreasing glucose concentrations might be ascribed to a decrease in the general condition of fish in our experiments. The significant relation found between rank of sampling and cortisol and glucose indicated a certain effect of the experimental procedure. However, this sampling effect must have had a limited impact because it

explained only 7% of the cortisol variation at 28‰ salinity and 7% of the glucose variation in spring at 7‰ salinity.

The aquaculture of marine fishes is expanding in northern countries. Several new species are being considered, including Atlantic cod. Declining landings have stimulated several attempts to grow cod in sea cages. Small cod selected from coastal gear landings are being reared in on-growing facilities in eastern Canada. Such installations require surface waters during summer or surface waters and groundwaters for fish production all year round. Both waters are brackish to various degrees, depending on location, but groundwater has the advantage of being warmer during freeze-up in winter. This study showed that cod can tolerate salinities down to 7‰. Rearing cod in estuaries or using groundwater in coastal regions would thus appear to be feasible provided that cod maintain or increase their potential for growth under such conditions.

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Physiological Effects of Brief Air Exposure in Exhaustively Exercised Rainbow Trout (*Oncorhynchus mykiss*): Implications for "Catch and Release" Fisheries

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Ferguson, R. A., and B. L. Tufts. 1992. Physiological effects of brief air exposure in exhaustively exercised rainbow trout (*Oncorhynchus mykiss*): implications for "catch and release" fisheries. *Can. J. Fish. Aquat. Sci.* 49: 1157–1162.

Rainbow trout (*Oncorhynchus mykiss*) which were air exposed for 60 s after exhaustive exercise initially had a much larger extracellular acidosis than trout which were only exercised. In both groups, however, plasma pH returned to normal by 4 h. Blood lactate concentrations were also greater in the air-exposed fish and continued to increase throughout the experiment. During air exposure, there was retention of carbon dioxide in the blood, and oxygen tension (P_{O_2}) and hemoglobin:oxygen carriage ($Hb:O_2$) both fell by over 80%. After 30 min of recovery, however, blood gases resembled those in fish which were only exercised. Finally, survival after 12 h was 10% in control fish and 88% in the exercised fish but fell to 62 and 28% in fish which were air exposed for 30 and 60 s, respectively, after exercise. These results indicate that the brief period of air exposure which occurs in many "catch and release" fisheries is a significant additional stress which may ultimately influence whether a released fish survives.

Des truites arc-en-ciel (*Oncorhynchus mykiss*) exposées à l'air pendant 60 s après une activité physique épuisante ont présenté, tout d'abord, une acidose extracellulaire beaucoup plus élevée que celles qui n'avaient pas été exposées à l'air. Dans les deux groupes, toutefois, le pH plasmatique est revenu à la normale dans les quatre heures suivantes. La concentration de lactate sanguin était également plus élevée chez les poissons exposés à l'air, et elle a continué à augmenter tout au long de l'expérience. Pendant l'exposition à l'air, on a enregistré une rétention de dioxyde de carbone dans le sang, la tension en oxygène (P_{O_2}) et le transport de la molécule d'hémoglobine oxygénée ($Hb:O_2$) diminuant tous deux de plus de 80%. Cependant, après 30 min de repos, les gas sanguins se situaient à des niveaux comparables à ceux des poissons n'ayant pas été exposés à l'air. Finalement, le taux de survie après 12 h était de 100% chez les témoins et de 88% chez les poissons ayant été soumis à des exercices épuisants, mais il a chuté à 62 et à 28% chez ceux qui ont été exposés à l'air pendant 30 et 60 s respectivement après ces exercices. Ces résultats indiquent que, dans de nombreux cas de pêche avec remise à l'eau des prises, la courte période pendant laquelle les truites sont exposées à l'air représente un stress supplémentaire important, qui peut en fin de compte, avoir une incidence sur la survie des poissons relâchés.

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An integral component of the management strategy in commercial and recreational fisheries is the release of a significant portion of the catch. In commercial fisheries, this may include species caught out of season or individuals which do not meet size restrictions. In recreational fisheries, "catch and release" policies have also been implemented to offset the impact of increased angling pressure on limited fish stocks. For example, recreational fishermen on Canada's east coast must now release all multi-sea-winter salmon (i.e. over 63 cm in length) and all smaller salmon over and above the daily or seasonal limit. Similar legislative restrictions apply to a diversity of species throughout North America (Barnhart 1989). Furthermore, in a number of sport fisheries, individuals as well as tournament organizers are promoting the live release of fish even in the absence of legislation.

Fish caught either by commercial or recreational methods often struggle to the point of complete exhaustion. Black (1957a, 1957b, 1957c, 1958) has shown that a significant percentage of these fish may die from the ordeal. Death does not

occur immediately, but often well into the recovery period. While other investigators have documented similar mortalities in exhaustively exercised fish (Bouck and Ball 1966; Beggs et al. 1980; Graham et al. 1982; Wood et al. 1983), some studies indicate that exhaustive exercise is not associated with significant mortality (Wydoski et al. 1976; Tufts et al. 1991). It is clear nonetheless that the period of exhaustive exercise associated with angling or struggling in commercial fishing gear results in a significant physiological disturbance in a fish (Wood and Perry 1985). Furthermore, complete recovery is not guaranteed simply because the exhausted fish is eventually released.

In both commercial and recreational fisheries, exhaustive exercise is often followed by a brief period of air exposure prior to release. During this time, the gill's delicate lamellae will collapse and gas exchange may be largely inhibited. The importance of this additional stress on the disturbance associated with exhaustive exercise and on the process of recovery has not previously been investigated. The purpose of the present study was therefore to examine the additive effect of brief air exposure on the physiological disturbance associated with exhaustive exercise in the rainbow trout (*Oncorhynchus mykiss*). In view

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of the rapidly growing importance of catch and release policies in the management of fisheries, it is hoped that this study will provide some insight into an additional stress which has often been overlooked, but which may ultimately influence the survival of released fish.

Methods

Animals

Freshwater-adapted rainbow trout (300- to 500-g males and females) were obtained from a local supplier and were maintained for at least 1 mo prior to experiments in dechlorinated Kingston tap water (8–10°C). The animals were fed to satiation every other day with commercially prepared trout pellets. At least 2 d prior to surgery, feeding was halted and the animals were transferred to an acclimation tank at the experimental temperature (15°C).

Surgery

The fish were anaesthetized in a 3-aminobenzoic acid ethyl ester (MS-222, Sigma) – NaHCO_3 – dechlorinated tap water mixture (1 : 2 : 10 000, w/w) prior to surgery. During surgery, the gills were continuously irrigated with a MS-222– NaHCO_3 mixture (10^{-1} the original concentration). The dorsal aorta was cannulated with PE 50 tubing (Clay Adams) filled with heparinized ($20 \text{ U} \cdot \text{mL}^{-1}$, Sigma) freshwater teleost saline (Hoar and Hickman 1983). Following the 5- to 10-min surgical procedure, animals were revived and placed in blackened perspex chambers with flowing dechlorinated tap water (15°C) where they remained for 24–48 h of recovery. This procedure allows the collection of dorsal aortic blood from unrestrained animals (Smith and Bell 1964).

Experimental Protocol

Following recovery, an 800- μL blood sample was removed from the resting fish with a Hamilton gas-tight syringe. The fish was then moved to a cylindrical tank where it was exhaustively exercised by manual chasing. After about 10 min, the fish would no longer respond to chasing and the exercise period was terminated. At this point, another blood sample was removed and the fish was returned to the blackened perspex box. Additional blood samples were taken 30, 60, and 240 min after exercise. A second group of fish was subjected to a similar protocol. However, these fish were moved to a damp cloth for 60 s immediately following the exercise period and the second blood sample was removed at the end of this brief period of air exposure. Finally a third group of control fish was subjected to a similar blood sampling procedure but was not exercised or air exposed.

Survival of fish was recorded after 12 h. For this data set, an additional group of fish was exercised and air exposed for 30 s but no blood samples were taken.

Analyses

True plasma pH (extracellular pH, pH_e) was determined with a PHM 73 pH meter and associated micro-pH unit (Radiometer, Copenhagen, Denmark) thermostatted to 15°C. Blood plasma was separated from the corpuscular component by 2 min of centrifugation in an Eppendorf centrifuge. Oxygen partial pressure in whole blood (PO_2) was measured with an E5046 oxygen electrode (Radiometer) thermostatted to 15°C and an associated oxygen meter (Cameron Instrument Co., Texas,

USA). A similar oxygen electrode was used to determine the total oxygen content (TO_2) of blood by the method of Tucker (1967). Total carbon dioxide contents (TCO_2) of whole blood and plasma were measured with a Corning model 965 CO_2 analyzer (CIBA Corning Canada Inc.). Hemoglobin (Hb) content of blood samples was measured by Drabkin's method using Sigma reagents and procedures (Sigma Bulletin No. 525). Whole-blood lactate concentrations were determined on neutralized perchloric acid extracts by the method of Lowry and Passonneau (1972). Measured values of true plasma total carbon dioxide and pH_e were used to calculate PCO_2 and true plasma bicarbonate concentration ($[\text{HCO}_3^-]_{\text{tp},1}$) via a rearrangement of the Henderson–Hasselbach equation with the values for pK' and αCO_2 determined according to Boutilier et al. (1984). The concentration of metabolic protons added to the plasma ($\Delta[\text{H}^+]_m$) over any given time period (e.g. time 1 to 2) was calculated according to McDonald et al. (1989) using the following equation:

$$\Delta[\text{H}^+]_m = [\text{HCO}_3^-]_{\text{tp},1} - [\text{HCO}_3^-]_{\text{tp},2} - \beta(\text{pH}_{e,1} - \text{pH}_{e,2})$$

where β is the nonbicarbonate buffer value of true plasma.

Statistics

Two sample Student *t*-tests (unpaired) were employed to determine the significance ($p < 0.05$) of differences observed between treatment groups. A one-way ANOVA was followed, where appropriate, by Dunnett's multiple comparisons test to determine significance ($p < 0.05$) between resting and recovery values within groups. All values are presented as the mean ± 1 SE.

Results

One minute of air exposure following exhaustive exercise promotes more severe acid–base disturbances than does exercise alone. In trout which were exercised, the pH_e fell by 0.239 pH unit whereas in trout which were also air exposed, the

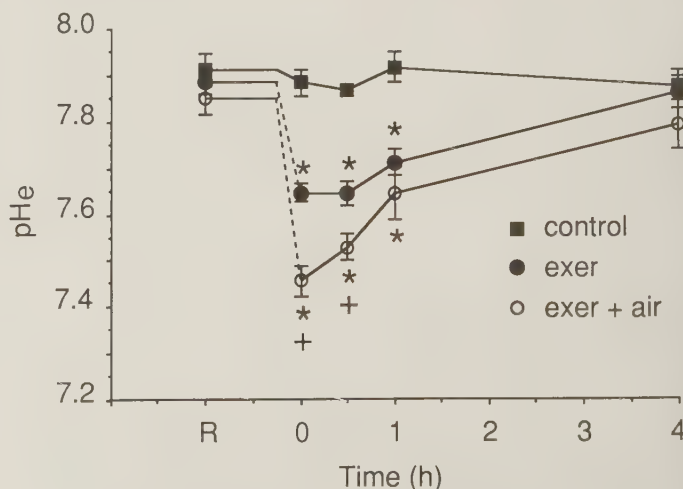


FIG. 1. Extracellular pH (pH_e) in rainbow trout at rest (R) and after 0, 0.5, 1, and 4 h under control conditions (■), following exhaustive exercise (●), or following exhaustive exercise plus 60 s of air exposure (○). Values are means $\pm$ SE (control, $N = 6$; exercise, $N = 8$; exercise + air, $N = 7$). An asterisk denotes a significant difference from the resting value. A plus sign denotes a significant difference between exercise and exercise + 60 s of air exposure.

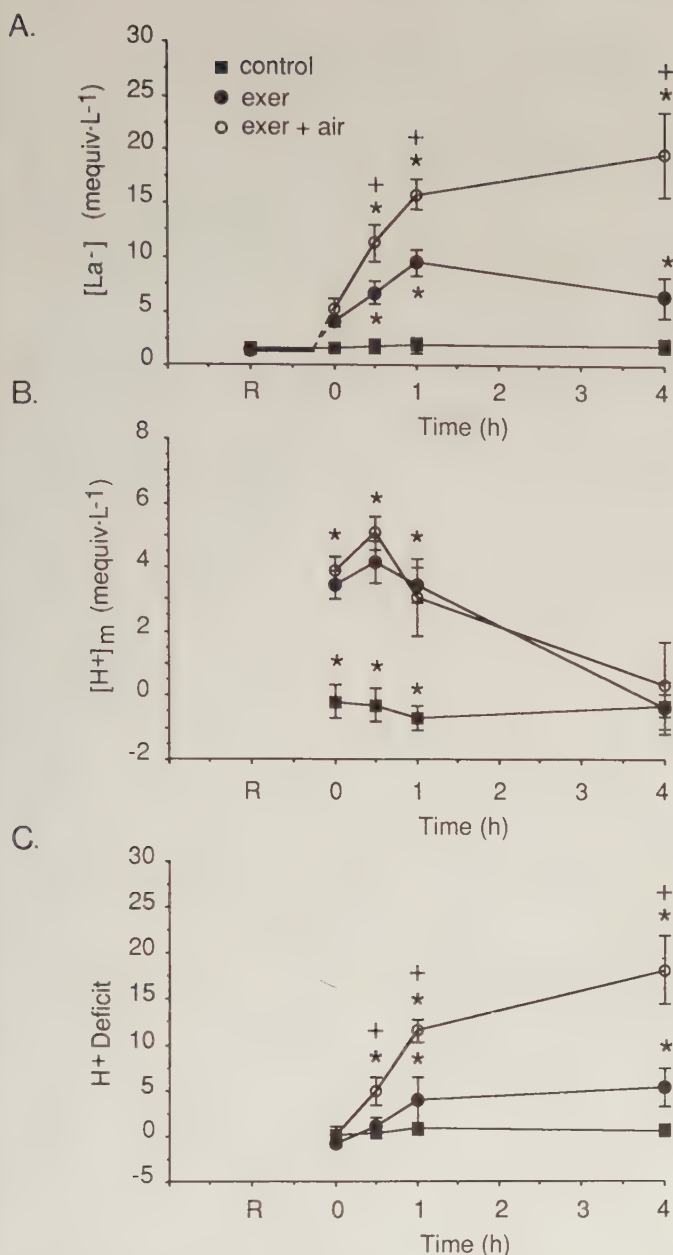


FIG. 2. (A) Blood lactate concentration ($[La^-]$), (B) metabolic proton load ($[H^+]_m$), and (C) proton (H^+) deficit in rainbow trout at rest (R) and after 0, 0.5, 1, and 4 h under control conditions (■), following exhaustive exercise (●), or following exhaustive exercise plus 60 s of air exposure (○). Values are means $\pm$ SE (control, $N = 6$; exercise, $N = 8$; exercise + air, $N = 7$). An asterisk denotes a significant difference from the resting value. A plus sign denotes a significant difference between exercise and exercise + 60 s of air exposure.

decrease in pH_e was 0.396 pH unit (Fig. 1). The pH_e remained significantly lower in the air-exposed group until the 1-h sample. By 4 h, the pH_e of both groups had returned to values which were no longer significantly different from those at rest.

A large increase in blood lactate was observed following exhaustive exercise as well as exhaustive exercise followed by brief air exposure (Fig. 2A). In the latter, the amount of lactate accumulated was significantly greater than that seen following exercise alone. The peak blood lactate in the exercise group was 9.6 ± 1.3 mequiv·L⁻¹ and occurred after 1 h of recovery. In contrast, the blood lactate in the air-exposed fish continued to increase throughout the experiment and the maximal concentration of 19.8 ± 3.9 mequiv·L⁻¹ was observed at 4 h.

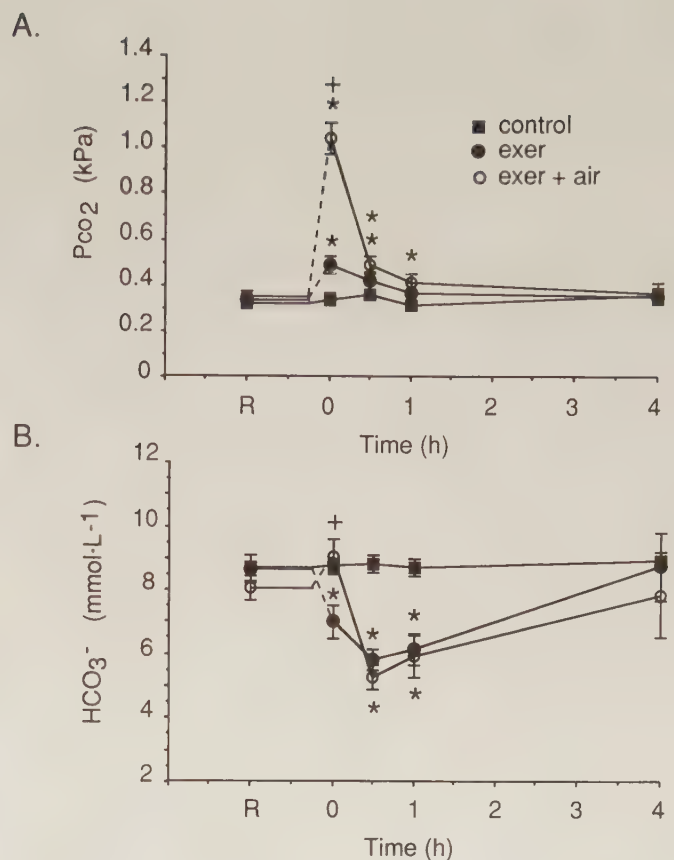


FIG. 3. (A) CO_2 tension (P_{CO_2}) and (B) bicarbonate concentration ($[HCO_3^-]$) in rainbow trout at rest (R) and after 0, 0.5, 1, and 4 h under control conditions (■), following exhaustive exercise (●), or following exhaustive exercise plus 60 s of air exposure (○). Values are means $\pm$ SE (control, $N = 6$; exercise, $N = 8$; exercise + air, $N = 7$). An asterisk denotes a significant difference from the resting value. A plus sign denotes a significant difference between exercise and exercise + 60 s of air exposure.

There were no significant differences in the blood metabolic proton loads of the two groups throughout the recovery period (Fig. 2B). Thus, due to the greater blood lactate concentrations, the air-exposed fish had a much greater proton deficit during the recovery period (Fig. 2C).

The acidosis following exercise also contains a respiratory component (Fig. 3). Although there is a significant reduction in plasma TCO_2 immediately after exercise, there is a 44% increase in P_{CO_2} (Fig. 3A). After air exposure, however, there is a significant increase in TCO_2 and a much greater increase (200%) in P_{CO_2} . Air exposure after exercise also causes a significant increase in plasma $[HCO_3^-]$ rather than the observed decrease in fish which were only exercised (Fig. 3B). Following 30 min of recovery, there were no longer any significant differences between these groups of fish, and by 4 h, these variables had returned to resting values.

Blood PO_2 was significantly reduced by 28% after exhaustive exercise, but there were no significant changes in hemoglobin:oxygen carriage ($Hb:O_2$) (Fig. 4). PO_2 returned to resting values after 1 h. In contrast, these variables were reduced by 82 and 87%, respectively, when the fish were also exposed to air for 1 min. Again, after 30 min of recovery in water, there were no significant differences between the two groups.

Exercise and brief exposure to air after exercise both had an impact on survival of the fish during the next 12 h (Fig. 5). Survival after 12 h ranged from 100% in the control fish to 28%

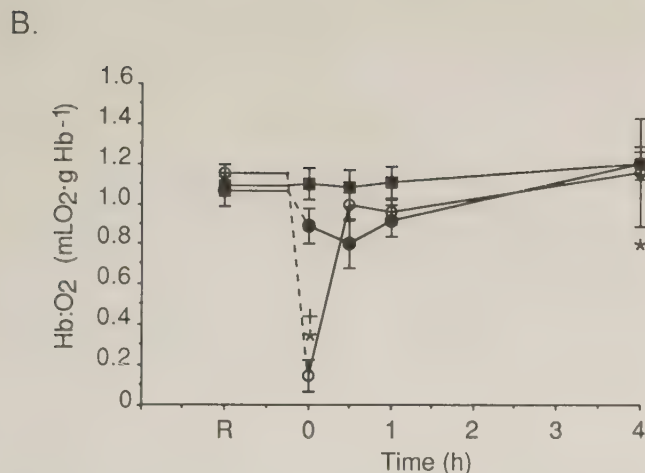
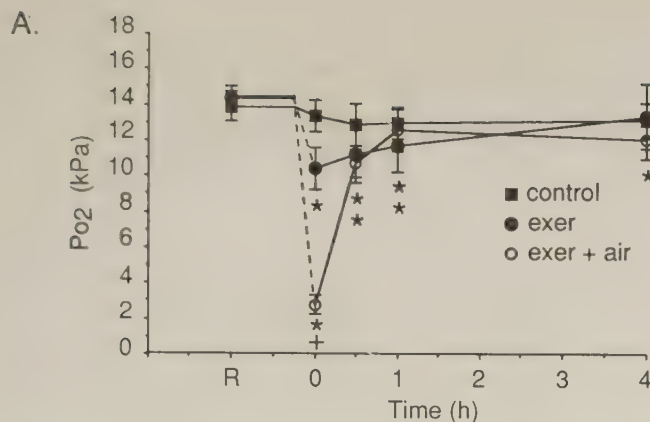


FIG. 4. (A) Blood oxygen tension (PO_2) and (B) hemoglobin:oxygen carriage ($Hb:O_2$) in rainbow trout at rest (R) and after 0, 0.5, 1, and 4 h under control conditions (■), following exhaustive exercise (●), or following exhaustive exercise plus 60 s of air exposure (○). Values are means $\pm$ SE (control, $N = 6$; exercise, $N = 8$; exercise + air, $N = 7$). An asterisk denotes a significant difference from the resting value. A plus sign denotes a significant difference between exercise and exercise + 60 s of air exposure.

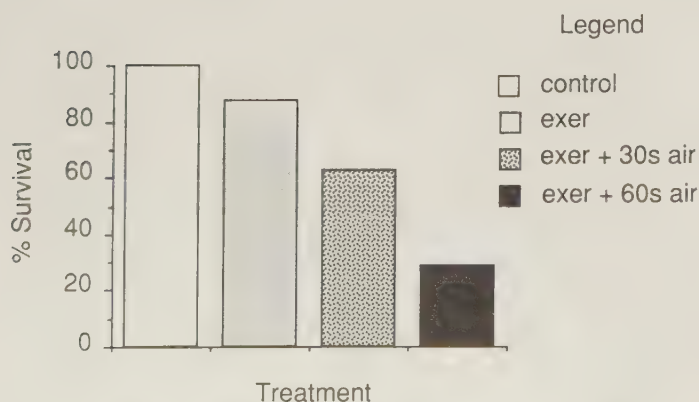


FIG. 5. Survival of rainbow trout 12 h following control conditions, exhaustive exercise, exhaustive exercise plus 30 s of air exposure, and exhaustive exercise plus 60 s of air exposure.

in the fish which were exposed to air for 60 s after exercise. When the period of air exposure was reduced to 30 s, survival increased to 62%, and in fish which were only exercised, survival was 88%.

Discussion

Physiological responses to exhaustive exercise have been well described in a number of fish species (e.g. reviewed in Wood and Perry 1985; Heisler 1986). The magnitude of the disturbance we observed in exhaustively exercised rainbow trout and the dynamics of the recovery process were very similar to that documented by previous investigators (Holeton et al. 1983; Turner et al. 1983; Milligan and Wood 1986; Primmitt et al. 1986; McDonald et al. 1989; Tang et al. 1989). However, the impact of brief air exposure on exhausted fish has not been examined previously; thus, the following discussion will focus primarily on this aspect.

In many commercial fisheries, fish which have struggled to exhaustion during capture are routinely exposed to air for sorting and identification prior to release. Similarly, in recreational fisheries, exhausted fish may be exposed to air for photos, measurements, or weighing before release. Indeed, this is common practice during angling contests and tournaments which have become very popular in recent years.

As demonstrated in previous studies, exhaustive or "burst" exercise in rainbow trout is associated with a considerable extracellular acidosis (Fig. 1). In fish which were also air exposed, the fall in plasma pH was much greater. After exhaustive exercise, the extracellular acidosis is normally composed of both a metabolic and a respiratory component (Wood and Perry 1985; Heisler 1986). The metabolic acidosis is caused by anaerobic production of lactate in the poorly perfused white muscle and subsequent release of the associated protons into the extracellular fluid. Our results indicate that the production of lactic acid is probably greater in fish which are briefly air exposed after exhaustive exercise (Fig. 2A). In the air-exposed fish, the blood lactate concentration was higher and it continued to increase throughout the experiment. Thus, even 60 s of air exposure following exhaustive exercise appears to cause a much greater degree of anaerobiosis within the muscle. Despite the higher plasma lactate concentrations, however, the metabolic proton load in the plasma of the air-exposed fish was not significantly different from that of the exercised group (Fig. 2B). Consequently, the proton deficit was larger in the air-exposed group. (Fig. 2C). This suggests either that there was a greater excretion rate of protons in the air-exposed fish after they were returned to water and/or that a significant portion of the metabolic proton load remained within the muscle. These two possibilities cannot be differentiated in the present experiments. However, according to Wood et al. (1983), mortality after exhaustive exercise is probably caused by the extent of the intracellular acidosis within the muscle. Thus, the increased mortality in the air-exposed fish may be evidence that a significant portion of these metabolic protons were retained within the muscle (Fig. 5). Clearly, further study into the muscle acid-base status of air-exposed fish is warranted and may explain the observed differences in survival.

In teleost fish, the majority of gas and ion transfer takes place across the delicate secondary lamellae which are aligned on the gill filaments. These lamellae are largely supported by the water flowing between them and, with few exceptions, the lamellae of most species will collapse if exposed to air (Boutilier 1990). Our results indicate that this reduction in respiratory surface area upon air exposure causes an almost complete inhibition of gas transfer across the gills in the rainbow trout (Fig. 3 and 4). In exhaustively exercised fish which remain in water, there is a significant reduction in the TCO_2 of plasma wherein bicarbonate is titrated by metabolic protons to form carbon dioxide

which is excreted (Fig. 3). This results in a transient increase in PCO_2 and a reduction in plasma bicarbonate after exhaustive exercise (Turner et al. 1983; Wood and Perry 1985; Heisler 1986; Milligan and Wood 1986; McDonald et al. 1989) (Fig. 3). However, in fish which are also air exposed following exhaustive exercise, TCO_2 significantly increases, indicating that gas transfer is largely inhibited while the animals are in air. The marked rise in blood PCO_2 during air exposure also explains the significantly greater reduction in blood pH (Fig. 1). Upon return to water, normal gill function is restored and the dynamics of carbon dioxide excretion are not significantly different between the two groups of fish (Fig. 3).

The large reduction in the oxygen content of blood during air exposure is possibly the most critical effect of exposing exhausted fish to air. This reduction was not attributable to any differences in hematocrit or total blood hemoglobin concentration but can be explained by the 81% reduction in PO_2 and associated 87% reduction in the amount of oxygen bound to hemoglobin after 60 s of air exposure (Fig. 4). The normal physiological responses of an exhaustively exercised fish combine to enhance oxygen transport to the tissues to compensate for the increased oxygen requirements immediately following exercise (Nikinmaa et al. 1984; Wood and Perry 1985; Primmatt et al. 1986; Milligan and Wood 1987). Our results indicate, however, that if the fish is even briefly exposed to air immediately after exhaustive exercise, the tissues will be temporarily deprived of oxygen during this critical period. Indeed, the large difference in plasma lactate concentration created by the air exposure is probably evidence of the detrimental effects of brief air exposure on the tissue metabolism of exhausted fish (Fig. 2A).

The issue of mortality in exhaustively exercised fish is an important management concern in catch and release fisheries. Black (1957a, 1957b, 1957c, 1958) originally demonstrated that significant mortality may occur in exhaustively exercised fish. Similar results have been obtained by a number of other investigators (Bouck and Ball 1966; Beggs et al. 1980; Graham et al. 1982; Wood et al. 1983). In contrast, there is also considerable evidence that mortality from exhaustive exercise is possibly very minimal in many catch and release fisheries (Wydoski et al. 1976; Barnhart 1989; Tufts et al. 1991). Differences in observed mortality may be due to a large number of variables which will undoubtedly be the focus of a great deal of study as the importance of both commercial and recreational catch and release fisheries continues to increase. In this regard, our study clearly demonstrates differences in mortality between exhaustively exercised fish and those which were also briefly exposed to air (Fig. 5). In fact, only 28% of those fish which were exposed to air for 60 s after exercise survived the next 12 h as compared with 88% of those fish which were only exercised. In each case, the extracellular acid-base status of the fish initially appeared to be returning toward normal, but the animals died between 4 and 12 h later. This "delayed mortality" has been observed by other investigators and, in the wild, could give the false impression that released fish always survive (Black 1958; Wood et al. 1983).

The purpose of the present experiments was not to predict actual percentages of mortality in the wild when exhausted fish are briefly exposed to air. The use of hatchery fish and repetitive blood sampling may have influenced our results in this regard. On the other hand, our results clearly indicate that the brief period of air exposure which commonly occurs in many catch and release fisheries is an important additional stress in an exhausted fish and may ultimately have a significant impact

on the number of released fish which survive. Finally, as the importance of catch and release fisheries continues to increase, fisheries managers may wish to place greater emphasis on the proper handling of exhausted fish.

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Mollusk Shell Growth: External Microgrowth Ridge Formation is Uncoupled to Environmental Factors in *Mytilus edulis*¹

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Bérard, H., E. Bourget, and M. Fréchette. 1992. Mollusk shell growth: external microgrowth ridge formation is uncoupled to environmental factors in *Mytilus edulis*. Can. J. Fish. Aquat. Sci. 49: 1163–1170.

Low-frequency (e.g. annual) external ridges have been used in the study of bivalve population dynamics, and it has been suggested that ridges produced at high frequencies are related to environmental factors. Using laboratory and field experiments, we examined the usefulness of fine external growth ridges as markers of cyclic growth patterns in the blue mussel, *Mytilus edulis*. Shells of *M. edulis* were analyzed to examine coherence of patterns of ridges on the shells. Analysis was carried out in two steps: (1) An objective method was developed to characterize the ridges by their height and thickness (precision 0.5 μm) and (2) laboratory and field experiments were carried out to determine the influence of environmental factors (immersion and emersion, algal concentration) and biological factors (valve closure) on the periodicity of growth ridge formation. All mussels exhibited unique growth ridge patterns. There was no concordance between growth ridge patterns of individual mussels grown together in the laboratory or in the field. These results refute the hypothesis of a similar growth ridge pattern between individuals in *Mytilus* at small scales. No relation was found between the mechanical closure of the valves and the formation of growth ridges.

Certains travaux de dynamique des populations faisant appel aux stries externes de basse fréquence sur la coquille des mollusques suggèrent que les stries de haute fréquence sont elles aussi reliées aux facteurs environnementaux. À l'aide d'expériences en laboratoire et sur le terrain nous examinons l'utilité des stries externes fines comme marqueurs des patrons cycliques de croissance. Nous analysons la cohérence entre les patrons de stries sur des coquilles de différents spécimens de *Mytilus edulis* maintenus dans des conditions expérimentales communes. Les analyses sont réalisées en deux temps : 1) nous développons d'abord une méthode pour caractériser les stries par leur hauteur et leur épaisseur (précision 0,5 μm) et 2) ensuite nous effectuons des expériences en laboratoire et sur le terrain pour déterminer l'influence des facteurs environnementaux (immersion et émergence, concentration algale) et des facteurs biologiques (fermeture des valves) sur la périodicité de formation des stries. L'analyse révèle que le patron des stries étudiées sont propres à chaque individu : nous n'observons aucune concordance entre les patrons individuels de stries de croissance chez les moules maintenues dans des conditions expérimentales communes, tant en laboratoire que sur le terrain. Ces résultats réfutent l'hypothèse d'une similitude des patrons de stries de croissance à petite échelle entre les individus de *Mytilus*. Aucune relation n'est observée entre la fermeture mécanique des valves et la formation des stries de croissance.

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Stress marks on the surface of invertebrate shells, such as those associated with the reduction of growth in winter, have long been used as annual markers (Lubinsky 1958; Le Gall 1970; Broom and Mason 1978; Peterson et al. 1983) and in the study of invertebrate population dynamics (Craig and Hallam 1963). However, studies using finer surface external growth ridges (defined here as external surface marks characterized by successive crests, each separated by a depression parallel to the margin of the shell; Bourget et al. 1991) have proven to be much more debatable. Davenport's (1938) observation that *Pecten irradians* produced four external growth ridges when left in a tank for 4 d supported the idea that invertebrate skeletons could be used to study the influence of daily environmental fluctuations on organisms. Expectations were raised by the report made by Wells (1963) that external growth

ridges on the epitheca of some corals appeared to correlate with the daily cycle and could be correlated also with the duration of the year. The diminishing numbers of external growth ridges found by Wells (1963) in Devonian corals (400 ridges), Carboniferous corals (380 ridges), and contemporaneous corals (360 ridges) were seen to be in reasonable agreement with the diminution of the year's duration associated with the known deceleration of the earth due to lunar tidal friction. It was this bold and stimulating claim which triggered a great deal of the research on the periodicity of shell growth structures (external surface growth ridges and internal growth bands).

External ridges have been reported to record cyclic annual (Craig and Hallam 1963; Peterson et al. 1983; Trutschler and Samtleben 1988), daily (Clark 1974; Broom and Mason 1978; Helm and Malouf 1983; Hurley et al. 1987; Joll 1988), and tidal (Le Gall 1970), noncyclic stochastic biological (Gruffydd 1981) or physical events (storms and gales, e.g. Lubinsky 1958; Clark 1974; but see Peterson and Ambrose 1984). At the small

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scale and depending on the species examined, less than one, one, or two external ridges per day were observed, suggesting that their formation may be in some way associated with daily or tidal events. In some studies, these relations were found to exist only over a short period of time, during the season of maximum growth, and not at other periods (Clark 1974; Broom and Mason 1978; Helm and Malouf 1983). Clark (1974) associated those periods of production of fewer external growth ridges with a cessation of growth. Published reports concerning growth ridges are usually based on average counts of these structures in many individuals and therefore do not always take into account individual variability in the form of missing or superfluous ridges (Bourget et al. 1991). In addition, growth ridges vary in height and width; hence, visual identification and enumeration can be influenced greatly by their perception by the observer.

In this paper, using the mollusk *Mytilus edulis* as a model animal, we address the usefulness of the small-scale (cyclic) patterns of ridges on the surface of the shell. Our approach was to compare patterns of external growth ridges between individuals, kept either in the laboratory or in the field. A tight coupling between growth ridge deposition and cyclic features of the environment (e.g. tidal or daily) should result in a high degree of similarity between patterns of cyclic ridges between individuals. Failure to meet this criterion should be considered as evidence against the hypothesis of detectable environmentally driven deposition of the ridges in *Mytilus*.

Materials and Methods

Our approach was to devise objective criteria for separating the different roughness elements at the surface of the shells (growth ridges) and to analyze, for each individual, the frequency of formation of the growth ridges, using spectral analysis (Trump and Bourget 1980). The shell profiles were obtained from mussels kept in the laboratory for 2–3 mo (see details below) and from mussels (same cohort, 1+ age, initial length 5–7 mm) grown in the field during 36 d, when growth was fastest. Thus, we first tested for the presence of periodic structures on the surface of each mussel, the alternative hypothesis being that the surface structures are not regularly spaced. Second, the ridge patterns were compared between mussels after standardizing total shell growth during the experiment to a nondimensional length of 1000 units for the 36-d experimental period. For each mussel, a nondimensional spatial scale was either contracted or expanded to ensure complete comparability between mussels. Since we used standardized spatial scales, any uniformity in temporal patterns of ridge formation should be reflected by a high degree of similarity in patterns among individuals. Lack of such similarity in standardized space is to be interpreted as a falsification of the hypothesis of detectable environmentally driven patterns of growth ridge deposition.

In another experiment in the laboratory, the valve movements were also recorded continuously to determine if the mechanical closure of the valves was associated with growth ridge formation.

Laboratory and Field Experiments

The individuals were acclimated to experimental conditions for 14 d in filtered seawater from the St. Lawrence Estuary ($11.0 \pm 0.1^\circ\text{C}$). In the laboratory, mussels were kept under constant conditions, either continuously immersed (controls) or periodically immersed and exposed according to a semidiurnal tidal cycle (4 h emersion : 8 h immersion), for periods lasting

from 64 to 86 d. Animals of each group were fed continuously on *Phaeodactylum tricornutum* concentrations of either 15 000 or 30 000 cells·mL⁻¹ in constant low light intensity. During emersion, air temperature was 11°C.

To test for the possible relation between mechanical closing of the valves and formation of a growth ridge, valve movements of two individuals were monitored continuously for 23 and 28 d each, using a Microswitch 924AB3W-L2P proximity sensor (sensitivity 0.035 mm). The proximity sensor recorded small changes in its magnetic field caused by the movements of an aluminum pin attached to the tip of a small plastic rod glued to the upper valve of the mussel. The lower valve was glued to a support to prevent lateral movement of the animal. Valve movements were recorded simultaneously on a chart recorder. One mussel was kept continuously immersed and the other exposed regularly 4 h every 12 h. The mussels were kept under constant conditions of air and water temperature of 11°C and fed with *P. tricornutum* (30 000 cells·mL⁻¹).

Field Experiments

To examine the spatial frequency of growth ridge formation, field experiments were carried out at Ste-Anne-des-Monts, Gulf of St. Lawrence, Québec, in a mixed semidiurnal tidal environment. Three groups of 20 individuals were placed in plastic mesh cages at three levels on the shore (continuously immersed, irregularly immersed and exposed at 0.65 m above chart datum, and regularly immersed and emersed at 1.1 m above chart datum, just below the upper limit of mussels in this area). The experiment lasted 36 d from 8 June to 14 July 1986.

Prior to laboratory and field experimentation, the exact location of the outermost margin of all the shells was clearly marked using epoxy glue (Fig. 1). At the end of each experiment, mussels were placed in a solution of concentrated 40% NaHClO₃ for approximately 10 min to remove the periostracum.

Shell Surface Profile Analysis

Continuous longitudinal surface profiles (perpendicular to the margin) of shell increment during the experimental periods were obtained using a DEKTAK II A surface profiler (2.5-μm diamond radius, vertical precision 10 Å (1 nm)) coupled with an IBM AT microcomputer. The precision of the shell profile measurements (data points were recorded at every 0.5 μm) was determined by comparing two transects of the same shell portion. Since “ridges” of different heights and thicknesses were observed on the shell, the precision of the measurements was studied for 10 different spatial scales. The series were first treated with mathematical filters (moving averages, Statgraphics statistical software) (STSC 1988) to extract from the series “ridges” spaced at intervals ranging from 0.5 to 2.5, 2.6 to 5.0, 5.1 to 7.5, 7.6 to 10, 10.1 to 12.5, 12.6 to 15, 15.1 to 20, 20.1 to 30, 30.1 to 50, and 50.1 to 75 μm. The statistical significance of the correlations obtained between the two measurements of the same shell profile was tested using a Monte Carlo permutation test for each of the 10 scales considered. One of the series was permuted at random 1000 times, giving rise, for each spatial scale, to a “random” data set. The correlations between the two adjacent series, for the 10 spatial scales considered, were all significantly different from their corresponding “random” data set at $P = 0.001$ (Hope 1968). Hence, the precision of the shell profile measurements was 0.5 μm (which corresponds to the initial step of data recording). To determine at what scales the ridges were continuous along the shell axis, two adjacent measurements (approximately 150 μm apart) of

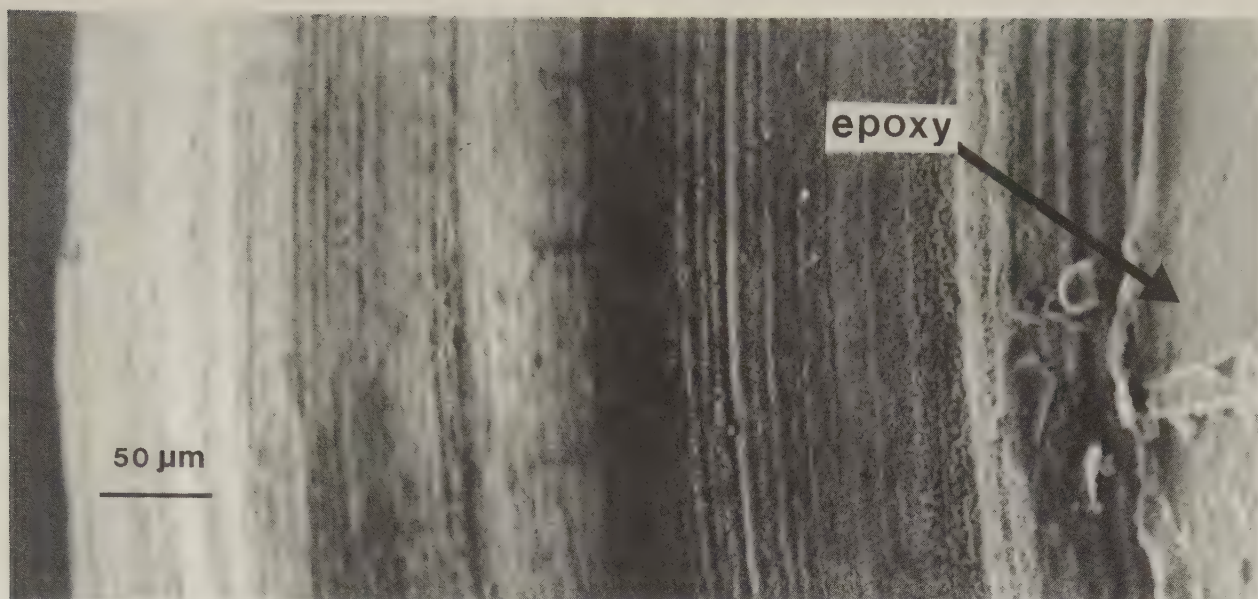


FIG. 1. Scanning electron micrograph showing the external ridges on the shell of *M. edulis* stripped of its periostracum. The epoxy mark indicates the beginning of the experiment on the shell.

the shell profile were compared for the 10 spatial scales considered. The significance of the correlations obtained was tested by performing a Monte Carlo permutation test as above. The correlations obtained between the two series were significantly different from their corresponding "random" data set at $P = 0.001$ for only the eight largest spatial scales studied (ridges ranging from 5.1 to 75 μm). Hence, "ridges" parallel to the margin of the shell, spaced at intervals greater than 5 μm , were considered to be continuous (at $P = 0.001$). "Ridges" spaced at intervals less than 5 μm could not be considered as growth ridges, since their presence was not continuous. They may represent the individual crystals which compose the growth ridge. Only data points spaced at intervals equal to 1 μm were retained for analysis of the shell profile, since this precision is sufficient to detect the smallest growth ridge (width 5 μm) while keeping the data files more manageable.

In spectral analysis, high frequencies can easily be masked by the presence of low frequencies. In order to circumvent this problem and facilitate the detection of a wide range of frequencies, it was decided to focus the analysis on three non-dimensional standardized spatial scales. According to a non-dimensional growth of 1000 units for a 36-d period (average daily growth rate = 27.7 length units $\cdot \text{d}^{-1}$), each spatial scale corresponded roughly to (1) less than a semidiurnal cycle of 12 h, for growth ridges formed at a periodicity less than 13.8 length units, (2) the semidiurnal component of the tidal cycle (≈ 12.4 h) and the daily cycle (24 h), for growth ridges formed at periodicities ranging from 13.8 to 27.7 length units, and (3) timescales greater than the daily (24 h) or lunar day (24.8 h), for other growth ridges formed at a periodicity greater than 27.7 length units. Mathematical filters were designed (BMDP statistical software, Parzen window, cosine shape) (Dixon 1983) to extract from the spatial series a range of periodicities corresponding to the different scales. The filters were built conservatively and with some overlap in order to cover the range of periodicities under study and to ensure that the signals kept were the least affected by the bias associated with the filter structure (Chatfield 1980). The bandwidths chosen for the bandpass filters were four times larger than the range of fre-

quencies under study. When using highpass or lowpass filters, the bandwidth was made equal to twice the range of frequencies under study, in order for the 0.5-point bandwidth of the filter to correspond to the frequencies to be removed (Priestly 1981). For a standardized growth of 1000 length units for a 36-d period, the filters used corresponded to (1) a highpass filter with a bandwidth of 0.144 (BMDP statistical software) to efficiently retain periodicities smaller than 13.8 length units, (2) a bandpass filter center at frequency 0.054 and with a bandwidth of 0.07 (BMDP software) which retains efficiently all periodicities ranging from 11 to 53 length units, and (3) a lowpass filter with a bandwidth of 0.069 (BMDP statistical software) to study ridges formed at periodicities greater than 27.7 length units. The filtered data were analyzed using spectral analysis to detect the presence of periodic patterns and to determine their relative importance. In order to be able to detect the frequencies of interest, the bandwidth of the spectral window (T) was chosen according to the relation $T > 2(f_1 - f_0)$, where f_1 and f_0 represent the frequencies of interest (Jenkins and Watts 1968). Confidence intervals based on the χ^2 test ($\chi^2 \alpha = 0.025$, $\chi^2 \beta = 0.975$), as described by Priestly (1981), were used to discriminate between the dominant frequencies shown in the spectral estimates. Since spectral estimates indicate only the proportion of the variance explained by a particular frequency, detailed observations of the filtered series were necessary to clarify the exact behaviour of the growth ridge pattern. This is particularly important when a broad peak is observed in the spectrum, e.g. the variance of the signal is explained by a wide range of frequencies. On the shell, it may indicate that the distance separating the growth ridges is continuously decreasing, continuously increasing, or varying over a certain range. Spectral analysis of spatial series requires stationarity of the first and second order in the series, e.g. no tendency in the data and homogeneity of the variance throughout the series (Laurec 1983). In our study the various major trends in the series are of interest and were kept in some analyses. The nonstationarity observed in the signal, e.g. the changes in height and spacing of the growth ridges, is of particular interest because of its possible relation with environmental factors. Our data were not normally distributed, but

TABLE 1. Total growth for field mussels kept at three levels on the shore at Sainte-Anne-des-Monts, Québec (A, continuously immersed; B, irregularly immersed and emersed; C, regularly immersed and emersed) during a 36-d (70 semidiurnal tidal cycles) experimental period. Mean growth rates were not significantly different between animals from the three shore levels (ANOVA, $P < 0.05$).

Mussel No.	No. of emersions	Total growth (μm)
A		
1	0	3033
2	0	2397
3	0	2508
B		
4	15	1897
5	15	3528
6	15	2711
7	15	2385
8	15	2558
C		
9	70	2703
10	70	1927
11	70	2136
12	70	2524
13	70	1704

TABLE 2. Total growth of laboratory mussels fed on two concentrations of *P. tricornutum* (A, 15 000 cells·mL⁻¹; B, 30 000 cells·mL⁻¹) for two conditions, continuously immersed or regularly immersed and emersed according to a semidiurnal tidal cycle (8 h immersion : 4 h emersion).

Mussel No.	Laboratory conditions	No. of days	Total growth
A			
1	Continuously immersed	64	2025
2	Continuously immersed	64	646
3	Regularly immersed	64	1630
B			
4	Continuously immersed	86	1300
5	Continuously immersed	66	710
6	Regularly immersed and emersed	66	948
7	Regularly immersed and emersed	66	886
8	Regularly immersed and emersed	66	1787

owing to the large number of data in each series (646–3033 data points), this is of no consequence (Platt and Denman 1975).

Results

Growth Rates

In field animals, large variations of total growth were observed between individuals within different groups (Table 1). Total growth was not significantly different between the three shore levels at $P = 0.05$ (ANOVA). In laboratory animals, total growth was extremely variable. For instance, mussel No. 1 grew three times as much as mussel No. 2, although both animals had similar experimental conditions throughout the experiment (Table 2).

TABLE 3. Analysis of external ridge patterns, occurring at periodicities ranging between 27.7 and 583.3 μm (mussels 1, 2, and 3), 27.7 and 611.1 μm (mussels 6, 7, and 8), or 27.7 and 805.5 μm (mussel 4) for a standardized growth rate of 27.7 $\mu\text{m} \cdot \text{d}^{-1}$. The mussels were (A) kept continuously immersed or (B) emersed regularly 4 h every 8 h of immersion and fed at two concentrations of *P. tricornutum* (*15 000 cells·mL⁻¹; **30 000 cells·mL⁻¹) during the experimental period. The periodicities shown are those found to be significantly different (based on χ^2 test at $\alpha = 0.975$ and $\beta = 0.025$) from the neighbouring ones.

Mussel No.	Periodicity (μm)	% of variance explained by each periodicity	Frequency of formation of ridges (ridges·d ⁻¹)
A			
1*	64.1–101.8	1.5	0.27–0.42
2*	111.1	0.4	0.25
	288.8–722.2	47.5	0.04–0.10
4**	238.9–319.8	0.6	0.09–0.12
	479.7–639.7	18.3	0.04–0.06
B			
6**	90.9–183.7	0.4	0.15–0.30
7**	146.9–184.3	0.03	0.15–0.18
	293.9–492.7	1.8	0.06–0.09
8**	244.9–293.9	9.0	0.09–0.11

Rate of Production of Growth Ridges

Growth ridges formed at periodicities greater than 27.7 length units

The longest period that can be studied in a series can be no more than one third the total length of the series (Priestly 1981). For the field mussels, this included only the growth ridges produced at periodicities ranging between 27.7 and 333.3 length units (12 d). For the laboratory mussels, the periodicities under study were between 27.7 and 583.3 length units (21 d), 611.1 length units (22 d), or 805.5 length units (29 d), depending on the mussel. Spectral analysis of the filtered data revealed that for these large spatial scales, the variance in the series is explained by a wide range of frequencies; hence, no single dominant periodic pattern was detected. Growth ridges were formed irregularly on the shells. For instance, in laboratory-maintained mussels, growth ridges occurred irregularly on the shells, at frequencies ranging from 0.04 to 0.42 growth ridge·d⁻¹ depending on the mussel (Table 3). Each individual had its own particular ridge pattern. Further, there was no apparent relationship between the frequency of growth ridge formation and the experimental conditions (immersed or emersed at the two feeding regimes). In the field experiment, the rate of formation of growth ridges ranged from 0.12 to 0.66 growth ridge·d⁻¹, depending on the mussel (Table 4). No apparent relationship was found between shore level and the frequency of formation of these growth ridges. In both laboratory and field conditions, there was a fairly low percentage of variance explained by these growth ridges in the raw data (Tables 3 and 4). The remaining unexplained variance in the raw data may be due to periodic patterns of growth ridge formation of longer periodicity (like those associated with the overall shape of the shell) or aperiodic occurrences of growth ridges.

Growth ridges produced at periodicities ranging from 13.8 to 27.7 length units

In the laboratory experiments, mussels continuously immersed produced growth ridges at frequencies ranging from

TABLE 4. Analysis of ridge patterns, occurring at periodicities ranging between 27.7 and 333.3 μm (for a standardized total growth of 1000 μm) on the shell of mussels kept at three levels on the shore (A, continuously immersed; B, irregularly immersed and emersed; C, regularly immersed and emersed) for a 36-d (70 semidiurnal tidal cycles) experimental period. The periodicities shown are those found to be significantly different (based on χ^2 test at $\alpha = 0.975$ and $\beta = 0.025$) from the neighbouring ones.

Mussel No.	Periodicity (μm)	% of variance explained by each periodicity	Frequency of formation of ridges (ridges $\cdot\text{d}^{-1}$)
A			
1	43.5–61.3	2.1	0.45–0.63
	133.8–200.1	4.4	0.13–0.21
2	46.7–66.7	6.0	0.42–0.59
	133.5	13.0	0.21
3	46.6–51.8	8.0	0.53–0.59
B			
4	65.9–76.9	0.3	0.36–0.42
	200.3	8.0	0.13
5	44.2–51.6	2.4	0.54–0.62
6	99.6–184.4	8.4	0.15–0.27
7	39.0–51.7	5.3	0.50–0.66
	78.0–93.7	14.0	0.27–0.33
8	50.0–66.8	0.9	0.41–0.55
	80.1–98.1	20.9	0.28–0.35
C			
9	51.4–77.3	6.2	0.36–0.54
	132.8–199.4	10.5	0.14–0.21
10	65.9–76.8	0.7	0.36–0.42
11	99.7–133.4	4.1	0.21–0.27
12	92.7–154.5	19.6	0.17–0.28
	202.85	17.4	0.12

TABLE 5. Analysis of external ridge patterns, occurring at periodicities ranging between 11 and 53 μm , for a standardized growth rate of 27.7 $\mu\text{m}\cdot\text{d}^{-1}$. The mussels were (A) kept continuously immersed or (B) emersed regularly 4 h every 8 h of immersion and fed at two concentrations of *P. tricornutum* (*15 000 cells $\cdot\text{mL}^{-1}$; **30 000 cells $\cdot\text{mL}^{-1}$) during the experimental period. The periodicities shown are those found to be significantly different (based on χ^2 test at $\alpha = 0.975$ and $\beta = 0.025$) from the neighbouring ones.

Mussel No.	Periodicity (μm)	% of variance explained by each periodicity	Frequency of formation of ridges (ridges $\cdot\text{d}^{-1}$)
A			
1*	16.7–33.4	78	0.8–1.6
2*	16.7–27.7	77	0.7–1.0
4**	18.4–25.7	58	1.1–1.7
B			
6**	15.5–27.1	86	1.0–1.8
7**	16.6–22.7	65	1.2–1.7
8**	19.4–23.5	61	1.2–1.4

TABLE 6. Analysis of ridge patterns, occurring at periodicities ranging between 11 and 53 μm (for a standardized total growth of 1000 μm) on the shell of mussels kept at three levels on the shore (A, continuously immersed; B, irregularly immersed and emersed; C, regularly immersed and emersed) for a 36-d (70 semidiurnal tidal cycles) experimental period. The dominant periodicities retained are those found to be significantly different (based on χ^2 test at $\alpha = 0.975$ and $\beta = 0.025$) from the neighbouring ones.

Mussel No.	Periodicity (μm)	% of variance explained by each periodicity	Frequency of formation of ridges (ridges $\cdot\text{d}^{-1}$)
A			
1	15.8	13	1.7
	19.8–29.0	56	0.9–1.4
2	13.3–22.1	56	1.2–2.1
	33.4	24	0.8
3	21.9–26.3	14	1.0–1.2
B			
4	16.8–21.1	57	1.3–1.6
5	18.4–27.5	71	1.0–1.5
6	17.7–23.9	59	1.1–1.5
	12.9	9	2.1
7	18.0–26.8	63	0.9–1.4
	16.4–18.8	35	1.5–1.6
8	26.6	28	1.1
C			
9	17.8–35.8	74	0.7–1.6
10	20.7–31.14	60	0.9–1.3
11	18.2–30.9	72	0.9–1.5
12	19.4	52	1.4

0.7 to 1.7 growth ridges $\cdot\text{d}^{-1}$. Those subjected to a tidal cycle produced growth ridges at frequencies ranging from 1.0 to 1.8 growth ridges $\cdot\text{d}^{-1}$ (Table 5). Thus, again each individual had its own pattern of ridge formation.

For field mussels, spectral analysis of the filtered data showed that the frequencies of growth ridge formation ranged between 0.7 and 2.1 growth ridges $\cdot\text{d}^{-1}$, depending on the mussel (Table 6). Independently of shore level, each mussel had its own unique pattern of ridge formation. Only one broad peak was detected in each of the spectral estimates of the filtered data for one mussel of group A, three mussels of group B, and three mussels of group C (see Table 6). For six of these seven mussels, 57–74% (depending on the mussel) of the variance in the filtered data was explained by a wide range of periodicities corresponding to growth ridges being formed at daily frequencies ranging from 0.7 to 1.6 growth ridges $\cdot\text{d}^{-1}$. On the shell, this was represented by growth ridges formed at nondimensional distances varying from 17.7 to 35.8 length units, depending on the mussel (see Table 5). For example, for mussel No. 10, the dominant periodicities represented ridges separated by distances varying between 20.7 and 31.1 length units; hence, there could be up to a 10.4-unit lag between two successive ridges (Table 6). In one mussel from the highest level, a 19.4-unit periodicity (growth ridges formed on average every 16.77 h = 1.4 growth ridges $\cdot\text{d}^{-1}$) alone explained 52% of the variance of the filtered series. This is relatively high, considering that the analysis included all periodic structures from 11 to 53 length units. It also shows, however, that growth ridges of interme-

diate size often occur aperiodically on the shell surface. For four mussels (Nos. 1, 2, 7, and 8, see Table 6), each spectral estimate of the spatial series representing the growth ridge patterns showed two separate, significant peaks. A narrow peak representing growth ridges formed at frequencies of 0.8, 1.1, 1.7, and 2.1 growth ridges·d⁻¹, depending on the mussel, explains a relatively small percentage of the variance of the signals (between 9 and 28%). The broadness of the second peak in the spectral estimates indicates that a large proportion of the variance (35–63%) of the growth ridge patterns is explained by a wide range of frequencies (growth ridges being formed at frequencies ranging from 0.9 to 1.4 growth ridges·d⁻¹ for two of the mussels and 1.2 to 2.1 and 1.5 to 1.6 growth ridges·d⁻¹ for the other two), depending on the mussel. Hence, the growth ridges are formed irregularly on the shell.

Of the 12 animals analyzed, all had unique patterns of ridge formation. Only one (mussel No. 7) produced growth ridges approximating a semidiurnal frequency. Another individual (mussel No. 8) produced ridges approximating semidiurnal and diurnal frequencies. Some growth ridges did occur regularly on the shell, but their presence was masked by other growth ridges occurring irregularly and explaining the largest proportion of the variance. Results show that there is no clear relationship between formation of growth ridges and semidiurnal and diurnal components of the tidal cycle or the daily cycle.

Growth ridges formed at periodicities less than 13.8 length units

Growth ridge formation in field animals occurred at frequencies ranging from 2.1 to 6.4 growth ridges·d⁻¹. No clear relationship was found between shore level and the frequency of formation of growth ridges, the range of frequencies of growth ridge formation being similar for animals of the three shore levels (Table 7). For most animals, the growth ridges were formed irregularly on the shell, the growth ridge patterns being best explained by a range of periodicities rather than a dominant one.

Due to the reduced growth rate in the laboratory animals, growth ridges occurring at a periodicity less than 13.8 length units, which approximates the average nondimensional growth over a 12-h period, would be expected to appear on the shell at intervals less than 7 μ m (before standardization) for four of the six experimental mussels. Since this is near the critical length of 5 μ m for insuring continuity of ridges across the shell, the analysis of these four mussels was not carried out further. For the two other mussels with higher growth rates, the spectral estimates showed no dominant frequencies in the filtered data, the variance of the signals being spread over a wide range of frequencies, similar for both animals. Growth ridges were formed at distances ranging from 5 to 14 length units.

Valve Movements and Growth Ridge Formation

Tightly closed valves could enable the animal to redistribute the new shell material or to modify the position of the periostracum at the margin of the shell, which constitutes the mold onto which the new shell material is deposited. To test this hypothesis, valve movements were recorded on two mussels, one being immersed continuously the other being immersed and emersed regularly. For these two mussels, the shell valves were closed, respectively, 3.28 and 13.35% of the total experimental period. The most frequent duration of valve closure (156 and 238 times, respectively, for the mussel immersed continuously and the mussel immersed and emersed regularly) was equal to or less than 1 min (Fig. 2). The number and the position of the

TABLE 7. Analysis of ridge patterns, occurring at periodicities less than 13.8 μ m (for a standardized total growth of 1000 μ m) on the shell of mussels kept at three levels on the shore (A, continuously immersed; B, irregularly immersed and emersed; C, regularly immersed and emersed) for a 36-d (70 semidiurnal tidal cycles) experimental period. The periodicities shown are those found to be significantly different (based on χ^2 test at $\alpha = 0.975$ and $\beta = 0.025$) from the neighbouring ones.

Mussel No.	Periodicity (μ m)	% of variance explained by each periodicity	Frequency of formation of ridges (ridges·d ⁻¹)
A			
1	4.3–5.6	27	4.9–6.4
	7.3	31	3.8
2	6.7–7.5	24	3.6–4.1
	8.7–11.7	30	2.3–3.1
3	9.2	21	3.0
B			
4	8.9–12.6	52	2.2–3.1
5	5.6	12	4.9
	6.8–11.1	21	2.5–4.1
6	5.5–9.9	50	2.8–5.0
7	5.0–10.1	54	2.7–5.5
8	6.6–7.4	39	3.7–4.4
C			
9	4.8–5.2	14	5.3–5.7
	8.5–9.2	54	3.0–3.2
10	6.2–11.9	64	2.3–4.5
11	9.3–13.1	44	2.1–2.9
12	7.9–9.1	31	3.0–3.5

growth ridges produced did not fit the valve closure patterns of either mussel (Fig. 3).

Discussion

Excluding the study by Runcorn (1966), who attempted to identify external growth ridges on coral epitheca using photographic negatives, analyses of invertebrate growth ridges rely on visual identification and counting of ridges. The present study was undertaken because the authors were concerned that the basis for deciding what is a ridge is subject to interpretation and can lead to major errors.

In this study, the analysis of growth ridges on the surface of mussel shells, using objective methods, reveals different sizes of growth ridges, depending on the scale considered. Most growth ridges were produced at irregular intervals along the shell surface. Few growth ridges were produced at regular intervals. However, each mussel produced its own pattern of ridges at each of the three scales studied, none corresponding to some identifiable short-term cyclic feature of the environment. Such interindividual variability in the number of growth ridges has been noticed before in various taxa (Wells 1963; Clark 1974; Bourget and Crisp 1975; Broom and Mason 1978; Gruffydd 1981; Helm and Malouf 1983; Hurley et al. 1987), but these results, except for Bourget and Crisp (1975) and Gruffydd (1981), do not seem to have altered the perception that these growth ridges must in some way be associated with some environmental factor(s). Numbers of growth ridges greater than or

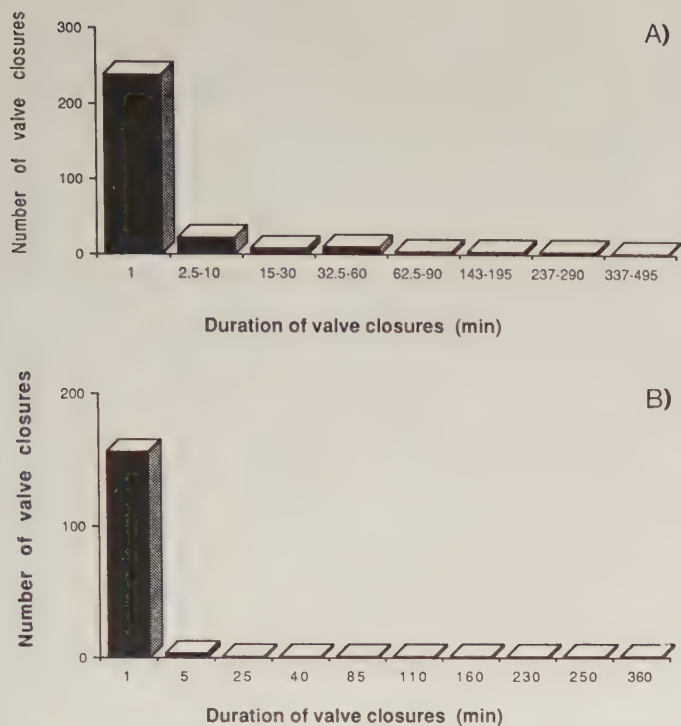


FIG. 2. Number and duration of valve closure of two laboratory mussels. (A) Mussel No. 4, kept in continuous immersion for 23 d in the laboratory; (B) mussel No. 6, regularly immersed and emersed (8 h immersion : 4 h emersion) for 28 d in the laboratory.

less than those expected have been assumed to result from various environmental stresses (temperature: Le Gall 1970; food availability: Broom and Mason 1978; heat stress, attack by predators: Clark 1974), but no evidence was presented to support this. The regularly immersed and emersed animals in the laboratory or animals grown in the field failed to show more meaningful environmentally controlled regularity in the production of growth ridges than our laboratory animals, grown under constant conditions of temperature, salinity, light, food, immersion, and total lack of heat stress, wave action, attack from predators, and other stochastic events.

Valve movements have been correlated positively with growth marks in *Mercenaria* (Thompson 1975). In *M. edulis*, no relationship was found between the pattern of valve movements and the pattern of external growth ridge formation, nor was there any regularity in the valve movement patterns.

It could be argued that some of the cycles observed in the series might approximate 1 growth ridge $\cdot d^{-1}$ (e.g. mussel No. 8, Table 6) and could be related to environmental cycles. However, unless this relation is demonstrated in many individuals, this result is no more than the individual response of the organism. In this context, it is evident that many interpretations of periodicity in the production of growth marks associated with tidal or diurnal variations, which originated from averaging individual periodicities approximating 1 or 2 ridges $\cdot d^{-1}$, are disputable. Departure from the expected 1 or 2 growth ridges $\cdot tide^{-1}$ or d^{-1} must be accounted for; otherwise, these results have to be considered as falsifying the hypothesis of cyclical formation according to a circatidal or circadiurnal rhythm (see Bourget et al. 1991).

In summary, the dominant growth ridge periodicities observed on the shells of *M. edulis* cannot be related to a dominant environmental periodicity. Further, each individual produced its own pattern of ridges. Therefore, we consider that our results

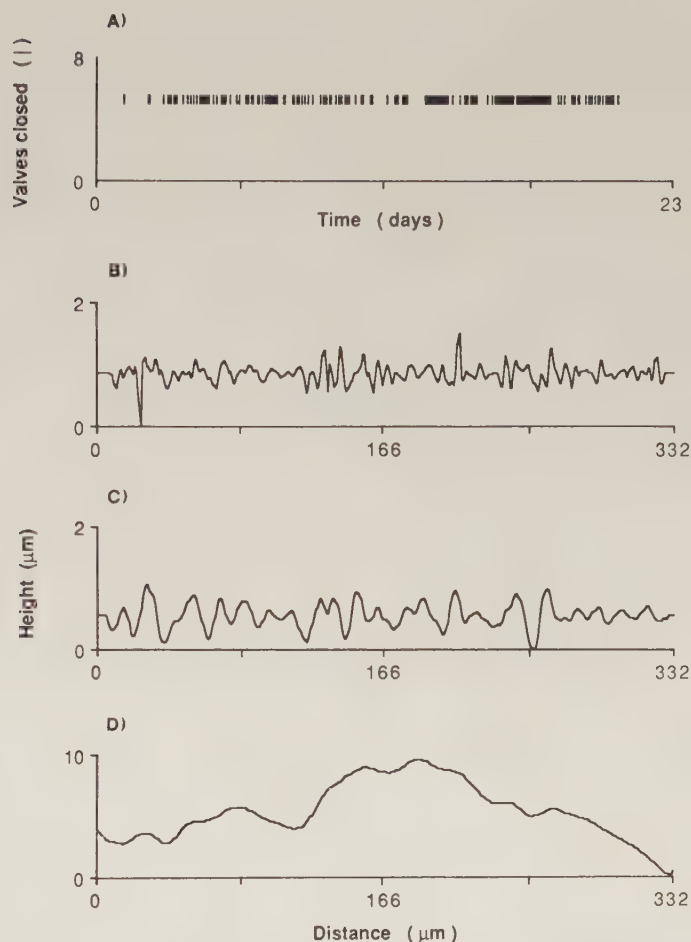


FIG. 3. (A) Temporal pattern of closure (bars) and opening (spaces) of the valves in a mussel (No. 4) continuously immersed during 23 d in relation to the growth ridge pattern occurring at (B) periodicities less than 12 h, (C) periodicities ranging from 10 to 50 h, and (D) periodicities greater than 24 h. Growth during the experimental period was estimated from the average daily growth rate over the 86 d ($11.5 \mu m \cdot d^{-1}$).

refute the hypothesis of environmentally driven detectable short-term ridge patterns in *M. edulis*.

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Relationship between Diatom Assemblages and Trophic Variables: A Comparison of Old and New Approaches

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Diatoms have been used as indicators of trophic status, and many ad hoc diatom indices have been developed. Although many of these indices have been useful, more direct methods for inferring trophic status are now available. Weighted-averaging regression and calibration was found to be a superior method for assessing lake trophic status from diatom assemblages when compared with a multiple linear regression index developed from a set of 30 Canadian Lakes.

Les diatomées servent d'indicateurs de l'état trophique, et l'on met au point de nombreux indices qui leur sont particuliers. Bien qu'un grand nombre de ces indices aient leur utilité, il existe maintenant des méthodes plus directes pour déterminer l'état trophique. En effet, la méthode de régression fondée sur les moyennes pondérées suivie de l'étalonnage s'est avérée supérieure pour évaluer l'état trophique d'un lac à partir d'assemblages de diatomées, par comparaison à l'indice de régression linéaire multiple qui a été mis au point d'après des études sur un groupe de 30 lacs du Canada.

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In the absence of long-term chemical monitoring, diatoms have been used to elucidate past environmental conditions in lakes (Dixit et al. 1992). A number of techniques have been used to infer lake water trophic status from sedimentary diatom remains, including indicator species (e.g. Bruggam 1988; Battarbee 1978), the Centrales:Pennales ratio (Nygaard 1956), and the Araphidineae:Centriceae ratio (Stockner 1971). More recently, multiple linear regression methods, which relate diatom distributions to lake trophic status, have been developed (Agbeti and Dickman 1989; Whitmore 1989). These methods are based on grouping diatom taxa into various categories, followed by the development of indices based on these categories. While the multiple linear regression models and indices are useful, they have some shortcomings (Milbrink 1983; Kansanen 1985; ter Braak and van Dam 1989; Birks et al. 1990b).

Paleolimnology as a quantitative science has developed rapidly over the past several years, and new statistical models have been developed largely due to research on lake acidification (ter Braak and van Dam 1989; Birks et al. 1990a, 1990b). These models, which include multivariate analysis and weighted-averaging (WA) regression and calibration, are superior techniques for assessing water quality than the use of indices, primarily because the estimates of species' parameters (e.g. optima and tolerances of taxa) are determined objectively (Kansanen et al. 1990). Ordination methods in assessing species distributions are more useful because they can detect simple structure in data by objectively reducing dimensionality (Gauch 1982). For example, using canonical correspondence analysis (CCA), Christie (1988) correlated limnological factors with the distribution of diatoms along a trophic gradient from the surface sediments of 39 lakes in southern Ontario. She found that diatom distributions were most closely related with nutrient con-

centrations (nitrogen and total phosphorus (TP)), chlorophyll *a* (Chl *a*), and lake depth.

The objective of this paper is to assess the advantage of CCA and WA regression and calibration over the use of indices. The paper indirectly compares the diatom inferred trophic index (DITI), a multiple linear regression index (Agbeti and Dickman 1989), with a multivariate (direct gradient) analysis (CCA ordination and WA regression and calibration). The reconstruction of environmental variables using WA calibration is compared with that of DITI using their coefficients of determination and the standard errors.

Method

The locations of the 30 lakes used to develop the DITI and concentrations of TP and Chl *a*, and Secchi depth measurements are presented in Table 1. In this paper, two of the lakes (Julian and Cataraqui Marsh) are omitted because their TP and Chl *a* data were not available. The sampling and diatom preparation and enumeration methods are described in Agbeti and Dickman (1989). Diatom taxonomy followed Cleve-Euler (1951–55), Hustedt (1930, 1939), Patrick and Reimer (1966, 1975), Gerloff and Cholnoky (1970), Bourelly (1981), Germain (1981), and Camburn et al. (1984–86).

DITI was derived using the formula

$$\text{DITI} = \frac{(\text{eut} + \text{meso} + \text{meso-eut} + \text{oligo-meso})}{(\text{oligo} + \text{meso} + \text{meso-eut} + \text{oligo-meso})}$$

where eut = the sum of eutrophic species, meso = the sum of mesotrophic species, oligo-meso = the sum of oligomesotrophic species, and meso-eut = the sum of mesoeutrophic spe-

TABLE 1. Lake number and name, location, and mean annual epilimnetic concentrations of TP and Chl *a* and Secchi depth measurements of 30 study lakes. Julian Lake and Cataraqui Marsh were omitted from the analysis because TP and Chl *a* values were not available.

Lake	Location (latitude, longitude)	TP (mg·m ⁻³)	Chl <i>a</i> (mg·m ⁻³)	Secchi (m)
1 Frederick	47°02'30"N, 80°37'30"W	2.0	0.3	5.0
2 Majorie	46°54'30"N, 80°37'30"W	5.0	0.4	6.5
3 Dougherty	46°59'05"N, 80°39'30"W	3.0	0.1	8.2
4 Bluesucker	47°10'00"N, 80°37'00"W	9.0	0.5	8.3
5 Pilgrim	47°10'00"N, 80°40'00"W	7.0	0.6	7.5
6 Chiniguchi	46°54'30"N, 80°42'30"W	3.0	0.1	8.5
7 Matagamasi	46°44'30"N, 80°36'30"W	8.0	0.7	5.0
8 Silvester	46°50'10"N, 80°38'30"W	2.0	0.2	6.8
9 Big Dam West	44°27'30"N, 65°18'30"W	11.1	1.7	1.4
10 Big Dam East	44°26'15"N, 65°19'40"W	7.9	1.2	4.2
11 Beaverskin	44°18'30"N, 65°19'40"W	4.7	1.4	6.8
12 Kejimikujik	44°22'30"N, 65°14'00"W	9.1	2.1	1.3
13 Pebbleloggitch	44°20'00"N, 65°21'00"W	10.0	1.6	1.6
14 B-Wawa	48°00'00"N, 84°51'40"W	6.5	1.9	9.0
15 Doc Crieg	47°29'15"N, 84°48'30"W	9.0	1.5	7.8
16 Crozier	47°54'10"N, 84°49'30"W	10.5	2.1	7.1
17 Kenessis	45°12'00"N, 78°35'00"W	10.6	1.9	7.5
18 12-Mile	45°02'00"N, 78°45'00"W	14.3	1.8	5.0
19 Stony	44°35'00"N, 78°05'00"W	17.0	4.3	3.5
20 Crawford	43°28'05"N, 79°57'00"W	16.5	3.9	4.6
21 Two-Mts.	45°31'00"N, 74°15'00"W	63.0	12.6	2.0
22 Upper Buckhorn	44°28'03"N, 78°22'30"W	24.5	8.8	2.3
23 Lower Buckhorn	44°33'00"N, 78°12'30"W	25.0	6.0	2.4
24 Chemung	44°25'00"N, 78°20'00"W	26.0	7.2	2.0
25 Pigeon	44°25'00"N, 78°30'00"W	27.0	4.3	2.1
26 Rice	44°10'00"N, 78°10'00"W	52.5	13.8	1.5
27 Scugog	44°07'30"N, 78°55'00"W	42.5	14.4	0.8
28 Julian	45°05'00"N, 78°55'00"W	—	—	3.0
29 Cataraqui	44°14'00"N, 76°28'00"W	—	—	3.8
30 Simcoe	44°25'00"N, 79°20'00"W	33.0	8.8	5.0

TABLE 2. List of common diatom taxa (occurring in at least three lakes), their number of occurrence, maximum and mean occurrences, and their weighted averages for TP, Chl *a*, and Secchi depth. WA values are presented as back-transformations of the log values of TP and Chl *a*.

Taxon	Number of occurrence	Maximum occurrence	Mean occurrence	TP (mg·m ⁻³)	Chl <i>a</i> (mg·m ⁻³)	Secchi (m)
1 <i>Achnanthes biasolettiana</i> (Kütz.) Grun.	4	2.8	0.33	22.44	4.90	2.3
2 <i>A. lanceolata</i> Bréb.	5	3.8	0.49	28.51	6.11	1.9
3 <i>Asterionella ralfsii</i> v. <i>americana</i> Körn.	4	32.5	1.27	7.91	1.69	7.2
4 <i>Cocconeis placentula</i> Ehr.	5	5.0	0.59	20.88	4.98	3.2
5 <i>Cyclotella comta</i> (Ehr.) Kütz.	9	29.3	5.72	6.92	1.07	6.6
6 <i>Eunotia pectinalis</i> (Kütz.) Rabh.	7	47.4	2.48	6.24	1.45	2.9
7 <i>E. pectinalis</i> v. <i>ventricosa</i> (Ehr.) Grun.	3	8.5	0.70	4.62	1.16	2.3
8 <i>E. serra</i> Ehr.	3	7.5	0.59	6.24	1.77	3.2
9 <i>Fragilaria acidobiontica</i> Charles	5	45.8	3.19	4.89	1.14	7.7
10 <i>F. brevistriata</i> Grun.	3	7.2	0.52	34.48	10.86	1.5
11 <i>F. construens</i> (Ehr.) Grun.	9	55.8	7.36	30.62	8.04	1.9
12 <i>F. constricta</i> Ehr.	5	54.8	6.95	4.01	0.55	5.3
13 <i>F. crotonensis</i> Kitton	3	22.0	1.21	10.22	2.44	4.8
14 <i>F. pinnata</i> Ehr.	7	12.3	1.35	26.54	6.52	2.0
15 <i>Frustulia rhomboides</i> v. <i>saxonica</i> Ehr.	3	4.0	0.42	6.24	0.66	5.9
16 <i>Melosira ambigua</i> (Grun.) O. Müll.	10	23.3	2.99	0.66	5.44	2.9
17 <i>M. distans</i> (Ehr.) Kütz.	8	11.0	1.48	0.91	2.08	6.1
18 <i>M. granulata</i> (Ehr.) Ralfs.	8	12.0	1.45	22.44	5.31	2.0
19 <i>M. lirata</i> (Ehr.) Kütz.	14	54.3	11.91	3.07	0.47	7.0
20 <i>M. lirata</i> v. <i>biseriata</i> (Grun) Camburn	4	11.8	0.71	3.27	0.46	7.3
21 <i>M. lirata</i> v. <i>lacustris</i> Grun.	5	8.0	1.00	4.37	0.67	8.6
22 <i>M. perglabra</i> Østr.	9	7.8	1.21	2.72	0.47	7.1
23 <i>M. sp. 9</i> (PIRLA)	4	4.7	0.45	33.67	8.95	1.8
24 <i>Pinnularia major</i> (Kütz.) Cl.	7	18.0	1.71	8.55	1.25	2.5
25 <i>P. viridis</i> (Nitzsch.) Ehr.	9	8.3	1.41	3.47	0.64	5.0
26 <i>Stephanodiscus</i> sp.	4	65.0	3.03	28.51	7.53	3.5
27 <i>Surirella linearis</i> W. Sm.	3	13.8	0.69	1.82	0.36	4.8
28 <i>Tabellaria flocculosa</i> v. <i>linearis</i> Koppen	4	4.0	0.42	17.20	4.79	4.3
29 <i>T. flocculosa</i> strain III <i>sensu</i> Koppen	5	32.5	1.81	8.55	1.90	6.6

cies. In all cases, eurytypic species and species with no trophic status data available in the literature were excluded from the equation. The details of the data are presented in Agbeti and Dickman (1989). Because Secchi depth showed a weak correlation with DITI, it was not considered in Agbeti and Dickman (1989), but has been included in the present study.

CCA was used to ordinate the species and sites using the computer program CANOCO, version 3.1 (ter Braak 1989, 1990). Forward selection and Monte Carlo permutation test (99 unrestricted permutations) options were used to identify the environmental variables that explain the maximum amount of variation in the species data. The raw data for Chl *a* were skewed and therefore, a log (Chl *a* + 1) transformation was used in order to satisfy the assumptions of CCA. Detrended correspondence analysis (DCA) was also used to ordinate the species in order to see if the selected variables account for a large proportion of the explainable variance in the species data.

WA regression and calibration, with classical deshrinking, were performed using the computer program WACALIB 2.1 (Line and Birks 1990) to derive inference models for TP and Secchi depth. WA regression and calibration is based on a species-packing model along an environmental gradient, assuming a unimodal distribution of the individual species along the environmental gradient of interest (ter Braak and Barendregt 1986; ter Braak and Looman 1986). An estimate of the species' optimum for an environmental variable is the average of all the values for the lakes in which the taxon occurs, weighted by the taxon's relative abundance. Conversely, an estimate of an environmental variable from a sample is the weighted average of the optima of all taxa present. In WA reconstructions, averages are taken twice (once in regression and once in calibration), and this results in a shrinkage of the range of inferred values. This is corrected using classical or inverse deshrinking (Birks et al. 1990b). I used classical deshrinking because this regression produced an even distribution of residual values.

Results and Discussion

The CCA ordination results of the diatom species and the three environmental variables show that TP and Secchi depth explain a large proportion (85%) of the species variance (Fig. 1A). Both TP and Chl *a* explain a large proportion of the variance in species distribution and they are highly correlated. TP was chosen for reconstruction over Chl *a* because TP is known to be an important variable in explaining variability in chlorophyll between lakes and reservoirs (Dillon et al. 1988). TP also has more direct use to lake managers. Although 82 species were used in the ordination, only the 29 most abundant species are presented (Table 2) in the biplot (Fig. 1A). The large angle between the vectors for TP and Secchi depth shows that these two variables are negatively correlated ($r = -0.68$), as expected. The CCA ordination biplot of the sample scores of the lakes in relation to the environmental variables (Fig. 1B) shows lakes of similar trophic status grouped together. The positions of Kejimikujik (26), Big Dam West (27), and Pebblelogitch (28) lakes may be attributed to the high colour values recorded for these lakes in comparison with other lakes in the region. Pebblelogitch, Kejimikujik, and Big Dam West lakes have colour values of 85, 65, and 70 Hazen units, respectively, whereas Big Dam East and Beaverskin lakes have 5 Hazen units each (Agbeti 1987).

The eigenvalues for the CCA axis 1 (0.77) and axis 2 (0.48) are similar to the eigenvalues of DCA axis 1 (0.91) and axis 2

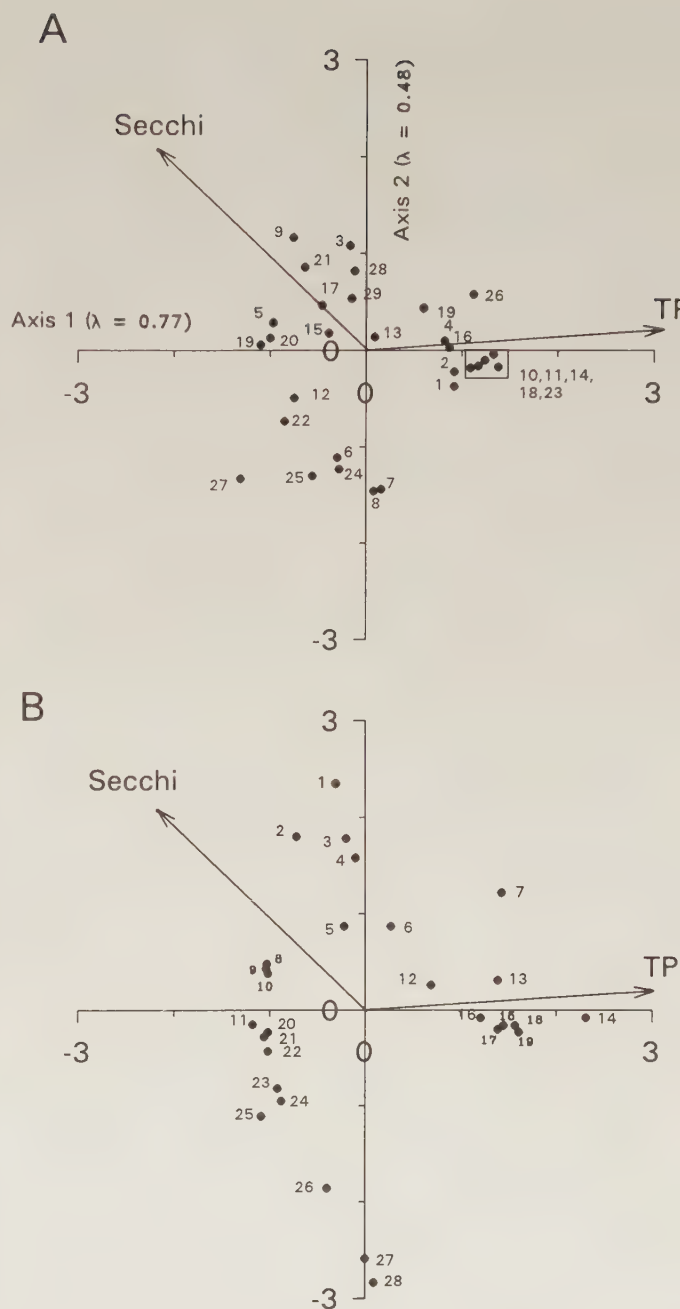


FIG. 1. (A) CCA ordination biplot showing the environmental variables (arrows) and diatom species (circles). The numbers represent the common diatom species (see Table 2). Taxa positioned on the left (i.e. 3, 5, 6, 9, 12, 15, 17, 19, 20, 21, 22, 24, 25, 27, 28, and 29) are more abundant in waters with high Secchi depth and low TP whereas taxa on the right (i.e. 1, 2, 4, 7, 8, 10, 11, 13, 14, 16, 18, 23, and 26) are more abundant in lakes with low Secchi depth and high TP. (B) CCA ordination biplot showing environmental variables (arrows) and sample scores of the study lakes (circles). The number correspond to the following lake names: 1, Doc Crieg; 2, Lake B; 3, Crozier; 4, Kennissis; 5, Twelve-Mile; 6, Crawford; 7, Simcoe; 8, Dougherty; 9, Chiniguchi; 10, Pilgrim; 11, Marjoie; 12, Stony; 13, Upper Buckhorn; 14, Two-Mts.; 15, Chemung; 16, Lower Buckhorn; 17, Pigeon; 18, Rice; 19, Scugog; 20, Beaverskin; 21, Silvester; 22, Bluesucker; 23, Big Dam East; 24, Matagamasi; 25, Frederick; 26, Kejimikujik; 27, Big Dam West; 28, Pebblelogitch. Lakes positioned on the upper left of the diagram (1, 2, 3, 4, 5, 8, 9, 10, 11, 20, 21, 22, 23, 25, and 24) have high Secchi depth and low TP corresponding to clear-water oligotrophic lakes. Lakes on the right half (7, 6, 12, 13, 14, 15, 16, 17, 18, and 19) have low Secchi depth and high TP corresponding to mesotrophic and eutrophic lakes.

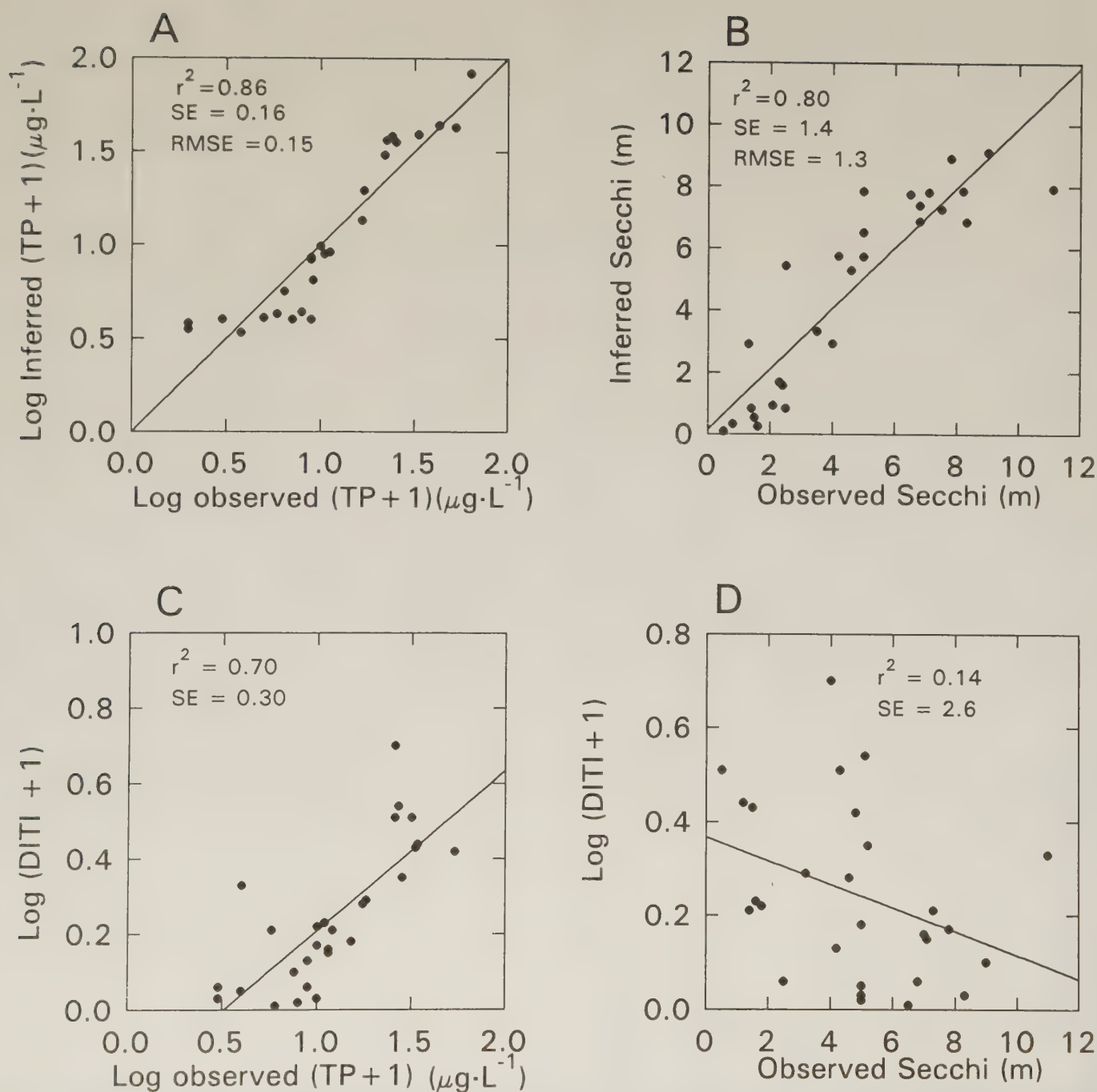


FIG. 2. The relationship between observed and WA diatom inferred (A) TP and (B) Secchi depth, and the relationship between observed and DITI inferred (C) TP and (D) Secchi depth. RMSE = root mean squared error; SE = standard error.

(0.80), suggesting that TP and Secchi depth explain a large proportion of the total variance in the diatom distribution. Both CCA axes are highly significant ($p < 0.01$). The two CCA axes explain 15% of the variance in the species data (9.2% of the variance being explained by the first axis and 5.8% by the second axis). A low amount of explained species variance is typical of noisy data sets with many zero values (Stevenson et al. 1991), and the low explained variance is not as important as the significance testing (ter Braak 1990). When CCA is constrained separately to TP, Secchi depth, and Chl *a*, the ratios of the eigenvalues of the first axis to the second axis are 0.89, 0.73, and 0.94, respectively, indicating that all three variables have a strong signal in the data set. Diatom distribution in lakes

is usually determined by many environmental variables in addition to those used in this study (e.g. Dixit et al. 1992). The eigenvalues of the CCA and DCA ordination axes may have been lower if more variables were available.

The correlations between observed and inferred TP and between observed and inferred Secchi depth are much greater for WA calibration than for DITI (Fig. 2), suggesting that the WA approach is superior. The residuals are more evenly spread around the 1:1 regression line for WA calibration than for DITI. The standard errors are lower for WA calibration than for DITI. Although Lake Simcoe was excluded from DITI because it was an outlier (Agbeti and Dickman 1989), it was not detected as an outlier in the WA calibration, suggesting that the new

approach is a more robust method.

The use of the DITI seems less satisfactory than direct gradient analysis (WA calibration) because (1) the categorization of the diatoms can be subjective and (2) only species for which trophic indicator status are available in the literature are used. However, there is an advantage of multiple regression of ecological groups over weighted averaging. Ecological data from sources other than those in a particular calibration can be used to calculate inferred values. Thus, if multiple regression is used, it is possible to infer past conditions for sediment intervals that contain data that were not included in the regional calibration. This is not possible with weighted averaging; however, an inferred variable using WA calibration is likely to be more reliable if the sample in question has close modern analogues with the species used for the calibration (see Birks et al. 1990b).

CCA has the advantage of allowing one to determine the proportion of variance among species that is explained by environmental variables. WA regression and calibration is superior to DITI because (1) a more theoretically sound community ecology model underlies the analyses, (2) there is no subjectivity in categorization of species, (3) all species are used, and therefore all information on species ecology is used, (4) inferences obtained are more easily interpreted when compared with index values, and (5) inferred absolute values of trophic variables are obtained which are more useful to lake managers. The results clearly demonstrate that the WA technique (ordination of species using CCA followed by WA regression and calibration) provides a more objective inference of trophic status than DITI.

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An Experimental Study of Piscivore–Planktivore Interactions: Population and Community Responses to Predation

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He, X., and R. A. Wright. 1992. An experimental study of piscivore–planktivore interactions: population and community responses to predation. *Can. J. Fish. Aquat. Sci.* 49: 1176–1183.

The population dynamics and behavior of an assemblage of fishes in a small bog lake were studied in a succession of whole-lake manipulations of piscivores. Total prey fish biomass declined after the addition of northern pike (*Esox lucius*). This decline was the result of emigration by cyprinid prey and consumption by northern pike. The emigration response of the cyprinids was dependent on cyprinid density. At high prey fish biomass, a significant portion of the loss in biomass was the result of emigration; this was not the case at low prey fish biomass. The prey fish community shifted from small-bodied soft-rayed species prior to the introduction of northern pike to species with spines or deep bodies after predator stocking. The community response was analyzed at four levels of numerical resolution: absolute, relative, and ranked abundance and presence–absence of prey species. High numerical resolution captured the dynamic short-term population responses to predation and suggests unstable community structure.

Le présente étude porte sur la dynamique et le comportement des assemblages de poissons vivant dans un petit lac-tourbière à la suite de modifications apportées à l'ensemble du lac en ce qui a trait aux piscivores. La biomasse totale des proies ichtyologiques a diminué après l'ajout de grands brochets (*Esox lucius*). Cette baisse résulte de l'émigration des proies ichtyologiques appartenant aux cyprinidés et de leur prédation par les grands brochets. L'émigration des cyprinidés s'est faite en fonction de la densité de ces derniers. Lorsqu'il y avait une forte biomasse de proies, l'émigration était la principale responsable de la diminution de la biomasse; cependant, la cause était tout autre lorsque la biomasse de proies ichtyologiques était faible. Avant l'introduction de grands brochets, la communauté de proies se composait d'espèces de petite taille et pourvues de rayons mous; après avoir empoisonné le lac de ces prédateurs, les proies ichtyologiques appartenaient à des espèces pourvues d'épines ou au corps épais. L'analyse de la réaction de la communauté de poissons s'est effectuée à quatre niveaux de résolution numérique: l'abondance absolue, l'abondance relative et le classement des espèces en fonction de l'abondance, ainsi que la présence ou l'absence de proies. La haute résolution numérique illustre bien les réactions dynamiques à court terme des populations face à la prédation et semble conclure à l'instabilité de la structure des communautés.

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He and Kitchell (1990) demonstrated the importance of indirect effects of predation on prey fishes in a 2-yr manipulation of the fish community in a small lake. Indirect effects are defined as habitat changes associated with predator avoidance behavior, increased emigration rates, and changes in the composition and size structure of the prey community. They showed that the decrease in prey fish biomass due to increased emigration was at least as great as that due to direct consumption by the predators. They also showed that predation caused significant changes in the prey fish community including decreased abundance of dominant species, increases in some rare species, and decreases in mean size of species most vulnerable to predation. This paper extends the data record on prey fish responses based on an additional 2 yr of experiments and also considers how the level of data resolution influences our ability to detect the impact of predators on the prey community. Data resolution refers to the analyses of changes in species abundance in terms of absolute abundance, relative abundance, ranked abundance, or presence–absence data.

The first part of the paper will focus on species-specific and behavioral responses of prey fish populations to the introduction of northern pike (*Esox lucius*) by testing the null hypothesis that direct (i.e. consumption of prey species) and indirect (i.e.

changes in migration rates and behavior) effects of predation are equally important.

In the second part of the paper, we focus on the qualitative effects of predation on the prey fish community. By using ordination analysis to explore and define patterns of the prey fish community and relate these patterns to predation effects, we argue that evaluation of the effects of predation on communities may depend on the level of numerical resolution at which a community is analyzed.

Materials and Methods

Study Site

The study site, Bolger Bog, is a small lake (area = 1 ha) located within the University of Notre Dame Environmental Research Center in Gogebic Co. in the upper peninsula of Michigan, USA. The fish community is diverse, consisting mainly of small planktivores and omnivores. No large piscivorous fishes are present in the lake (Table 1). Because the lake is shallow and is subject to winterkill, most fish species emigrate from the lake in late autumn and early winter and then return in spring through an outflow channel connected to a nearby stream. A more detailed description of the lake is presented in He and Kitchell (1990).

TABLE 1. Fish species caught in minnow traps in Bolger Bog, 1986–89.

Common name (abbreviation)	Scientific name	Length range (mm)
Northern redbelly dace (RB)	<i>Phoxinus eos</i>	45–85
Central mudminnow (MM)	<i>Umbra limi</i>	50–120
Fathead minnow (FM)	<i>Pimephales promelas</i>	50–90
Brook stickleback (SB)	<i>Culaea inconstans</i>	45–75
Finescale dace (FS)	<i>Phoxinus neogaeus</i>	42–90
Brassy minnow (BM)	<i>Hybognathus hankinsoni</i>	40–80
Golden shiner (GS)	<i>Notemigonus crysoleucas</i>	70–140
Blacknose shiner (BS)	<i>Notropis heterolepis</i>	50–75 ^a
Bluegill (SF) ^b	<i>Lepomis macrochirus</i>	30–60
Pumpkinseed (SF) ^b	<i>Lepomis gibbosus</i>	30–60
Creek chub (CC)	<i>Semotilus atromaculatus</i>	70–140 ^a
Yellow perch (YP)	<i>Perca flavescens</i>	90–140
Iowa darter ^c	<i>Etheostoma exile</i>	50–70 ^a
Pearl dace ^c	<i>Semotilus margarita</i>	70–140 ^a

^aEstimated^bPooled as sunfish in the analysis.^cVery rare.

Sampling and Experimental Design

The experiment was conducted from late May to late October from 1986 through 1989. Planktivorous fishes were sampled with minnow traps. Each sample was derived from six traps set in the channel, 12–26 traps set around the perimeter of the lake, and 9–18 traps set along transect lines in the open-water habitat. Fishes caught in each trap were identified to species, counted, and released. Densities of prey fishes were estimated by calibrating catch per unit effort (CPUE, number of fish caught per trap per hour) from minnow traps with mark-and-recapture methods (Petersen method, Ricker 1975). A detailed description of limnological and fish sampling is presented in He and Kitchell (1990).

The experiment was designed as an unreplicated paired-system experiment. The first year (1986) served as a baseline, the fish community in the absence of northern pike predation, for experimental manipulations in the following three years (1987–89). On May 23, 1987, 15 adult northern pike were introduced into the lake. The outflow channels were blocked by a wire fence of mesh size 2.5×2.5 cm², keeping northern pike in the lake but allowing prey fishes to pass through. On August 21, all northern pike were removed from the lake by gill net.

In June 1988, the lake was divided in half by a metal fence of 5×2.5 cm² mesh. This fence allowed passage of prey fishes but not of northern pike. Our intent was to produce a predator-free half of the lake as a refuge area for prey fishes. On June 6, 1988, eight adult northern pike (mean total length = 470 mm, SD = 15 mm) were released into the east side of the lake. From July 16 to 22, two gill nets were set on the east side of the lake to remove northern pike. The nets were checked each day and only four northern pike were caught from the east side. We assume that the other four northern pike died. After July 22, the lake was left pike-free until August 26 when 12 adult northern pike (mean total length = 474 mm, SD = 48 mm) were released into the west side of the lake. Those northern pike remained in the lake until the last sampling date (October 15) when only four northern pike were recovered. Length, weight, and stomach samples of each northern pike were taken upon removal.

On June 21, 1989, 12 northern pike (mean total length = 480 mm, SD = 44 mm) were released into the lake. Four northern pike were caught from the lake during the week of August 16 when four gill nets were set consecutively for 7 d.

Data Analysis

To examine year-to-year variability of prey densities, CPUE of individual species and all species combined were compared among the four years. Standard errors of CPUE for each sampling date were computed from all traps set on the same date. Randomized intervention analysis (RIA) (Carpenter et al. 1989) was used to compare the CPUE following the introduction of northern pike with the data prior to introduction (He and Kitchell 1990). The RIA method uses a series of observations from both reference system (i.e. outflow channel) and treatment system (i.e. lake) in premanipulation (i.e. northern pike absent) and postmanipulation (northern pike present). The absolute value of the difference between mean intersystem differences (D value) is the test statistic.

We used the emigration of prey fishes to quantitatively represent indirect effects. The value of R , the ratio of biomass change due to emigration to biomass change due to consumption by northern pike, was used to evaluate the relative importance of the indirect and direct effects of northern pike predation (He and Kitchell 1990). If R is greater than 1, northern pike predation has a greater indirect than direct effect whereas if R lies between 0 and 1, predation by northern pike has a greater direct than indirect effect. If R is less than 0, immigration may have occurred.

The data used in the community analysis are mean CPUE of individual species from all traps set in the lake from 1986 to 1989. For these data, a sampling unit was mean CPUE from each sampling date. A secondary data matrix was also created containing the environmental attributes of the samples in the main data matrix. The secondary matrix consisted of four factors: year, presence-absence of northern pike, day of year, and cumulative consumption of prey biomass by pike. The first two factors were treated as categorical variables, while the last two factors were treated as numerical variables. The year factor has four categories: year 1 (1986), year 2 (1987), year 3 (1988), and year 4 (1989). The northern pike factor has three categories: prepriperiod, pike period, and postpike period. The prepriperiod includes all sampling dates in 1986 and those sampling dates before the northern pike introduction for each year from 1987 to 1989. The pike period includes all sampling dates during 1987–89 after the introduction and before the removal of northern pike. The postpike period includes all sampling dates

during 1987–89 after removal of northern pike from the lake. The pike factor is divided into three categories instead of using only presence–absence so as to determine the rate at which the prey community recovers after northern pike removal. Since the prey fish community may take longer than several weeks to recover after removal of northern pike, it is necessary to include this time in the analyses.

The cumulative consumption of prey fish biomass by northern pike, estimated by a bioenergetics model for the growth and consumption of northern pike (Kitchell et al. 1977; Bevelhimer et al. 1985), was also included in the environmental matrix because it presents a numerical gradient of predation. Using the secondary matrix, we were able to numerically correlate environmental factors, including the northern pike treatments, with shifts in the prey community. We also overlaid the secondary matrix for a clearer graphical representation.

An indirect gradient analysis, detrended correspondence analysis (DCA) as recommended by Hill and Gauch (1980), Gauch (1982), Ludwig and Reynolds (1988), and Peet et al. (1988), was used in the analysis. DCA was then applied to absolute abundance (number of fish per trap per hour), relative abundance (percentage catch), and presence–absence data. The proportion catch data were normalized using an arcsine-square-root transformation (Sokal and Rohlf 1981) before the analysis:

$$Y_{ij} = \sin^{-1} (X_{ij})^{1/2}$$

where X_{ij} = proportion catch for species i at sample j .

Mean catch data also were transformed to ranked abundance and analyzed by multidimensional scaling (MDS) (Minchin 1987). Results from MDS were compared with those from DCA. Thus, four levels of observational scales were included in the analysis: absolute abundance, relative abundance, ranked abundance, and presence–absence of species.

To measure the stability of the prey fish community, contingency table analysis based on absolute abundances of prey fish and analysis of Kendall's coefficient of concordance (W) based on abundance rankings were used (Sokal and Rohlf 1981; Rahel 1990). In both analyses, three sampling dates from June, August, and October of each year were randomly chosen so that the total sample size over four years was 12. This was done to avoid an oversampling problem with limited degrees of freedom in the Kendall analysis ($df = 10$, total number of species = 11).

The multiresponse permutation procedure (MRPP) was used to determine if significant differences existed between groups in multidimension space (Zimmerman et al. 1985; McCune 1987). In the case of this analysis, the multidimension space is defined in species space. The MRPP procedure does not depend on normally distributed data or homogeneous variances. In the analysis, we separated the data on the ordination space into groups based on categorical factors in the secondary matrix, such as year (four years) and the northern pike treatment (three periods).

Correlations between ordination axes and species abundances or environmental factors were calculated to identify important species or environmental factors to determine the structure of the community. In testing significant correlations, a correlation coefficient r or tau $> |0.5|$ is considered ecologically significant unless otherwise specified. This preset critical value is rather arbitrary but is intended to mitigate the large-sample problem. When sample size (n) is large ($n = 55$ in this study), very small correlations may result in the rejection of the null hypothesis of correlation = 0 even though the true correlation is 0 (Zar 1984). When the critical value is set to be

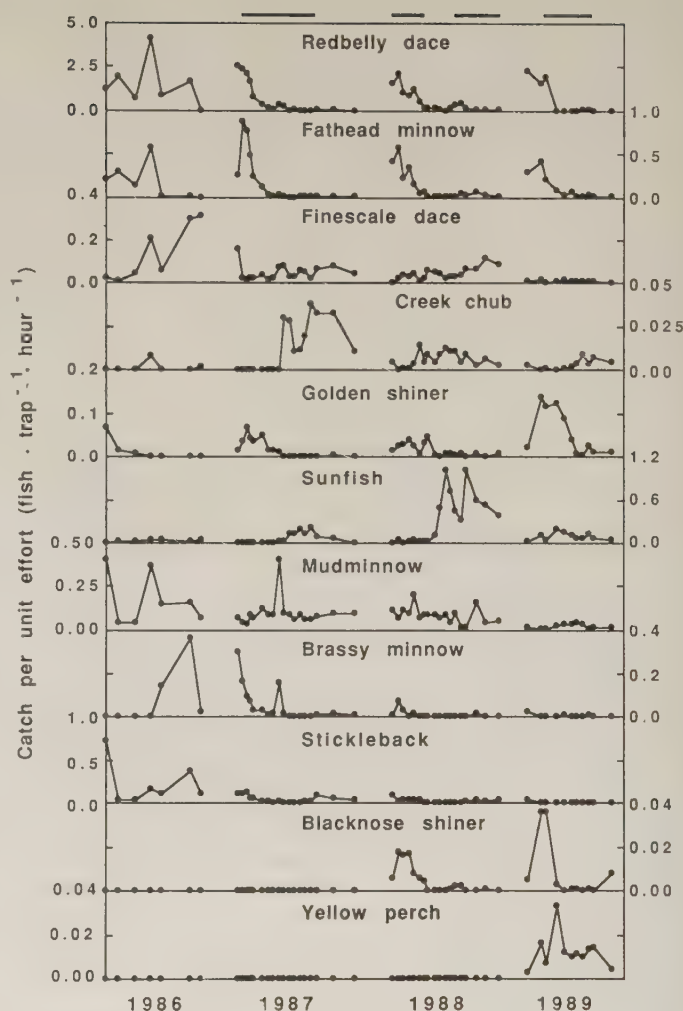


FIG. 1. Mean catch per trap hour (CPUE) of each species for Bolger Bog from 1986 through 1989. Periods when northern pike were present in the lake are indicated on the top of the upper panel by lines. Note that different scales are used on the y-axes.

0.5, fewer important species or environmental factors are selected in interpretation of the ordinations.

The ordination analyses were done using the PC-ORD program (McCune 1987) and SYSTAT (Wilkinson 1989). Procedures of DCA and MRPP are available in the PC-ORD program, and procedures of MDS, contingency table analysis, and Kendall's W are available in SYSTAT. The PC-ORD program is appropriate for ecological applications because of its ability to overlay the secondary matrix on the ordination space.

Results

The experimental design of the manipulation in 1988 and 1989 involved a comparison between the prey community on the side of the lake with northern pike versus the pike-free side. A complete analysis suggests that either the fence failed to keep northern pike on one side of the lake or that responses of prey fish to predation were at the scale of the whole lake (see He 1990 for this analysis). In this paper, we report the results assuming that the effect of northern pike was uniform across the entire lake.

Introduction of northern pike in three manipulated years (1987–89) significantly reduced total prey fish densities. The annual mean CPUE for all species of 1987, 1988, and 1989

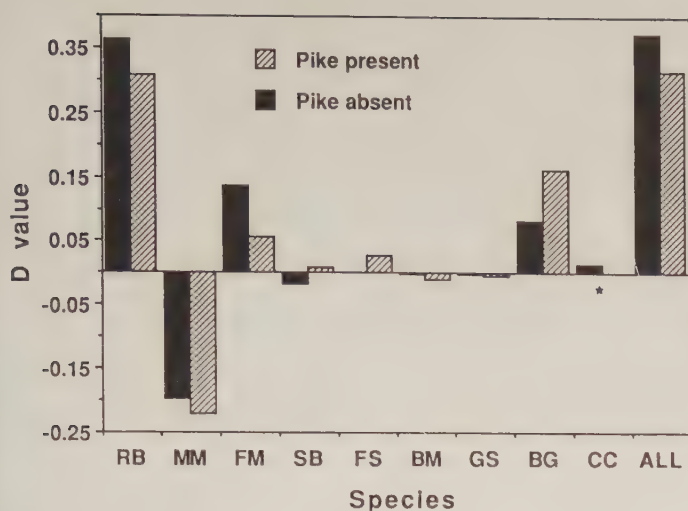


FIG. 2. Results of RIA for CPUE between outflow channel and lake perimeter in 1988 and 1989 combined. D values are differences of means between lake perimeter and outflow channel before (black) and after (cross-hatched) addition of northern pike. Positive D values indicate that fish prefer the lake perimeter, while negative D values indicate that fish prefer the outflow channel. The star indicates significant p values less than 0.05. See Table 1 for species abbreviations (ALL = all species combined).

was reduced to 47, 45, and 33% of that observed in 1986, respectively. Reduction in CPUE for prey fish was highly species specific. Catch was reduced for five species: northern redbelly dace, fathead minnow, finescale dace, brassy minnow, and brook stickleback, but catch increased for sunfish, golden shiner, and creek chub (Fig. 1) (see Table 1 for scientific names). Catches of central mudminnow did not show a clear difference. No comparison can be made for blacknose shiner and yellow perch because blacknose shiner appeared in the lake only in 1988 and 1989 and yellow perch only in 1989.

In all manipulated years, lake-wide CPUE declined for northern redbelly dace, fathead minnow, and finescale dace shortly after the introduction of northern pike, even though they were released into only one half of the lake in 1988 and 1989. Although the catches in early June 1989 were similar to those in early June 1988, CPUE declined later in 1989 than in 1988. This corresponded to later introduction of northern pike.

Total prey fish biomass at the beginning of 1988 and 1989 was 44 and 52%, respectively, of that in 1987, and total prey fish densities in 1988 and 1989 were 73 and 70% of that in 1987.

Removal of northern pike from the lake in the middle of summer was designed to release the predation pressure from the system. We expected that the catch of all species would increase due to immigration after the removal of northern pike. Catches increased during the pike-free period in 1988 (July 23 to August 26) but did not increase during the pike-free period in 1987 and 1989 (late August to October) (Fig. 1). This indicated that the immigration occurred primarily during July and August and stopped after August. The high catch in July 1986 also indicated active immigration in July.

The RIA test on 1986 and 1987 data showed that more northern redbelly dace, finescale dace, and brassy minnow shifted from the lake to the outflow channel after the addition of northern pike, and more sunfish shifted from the outflow channel to the lake (see Fig. 5, He and Kitchell 1990). The RIA test on data from subsequent years, 1988 and 1989, indicated that there was no significant shift between the outflow channel and the

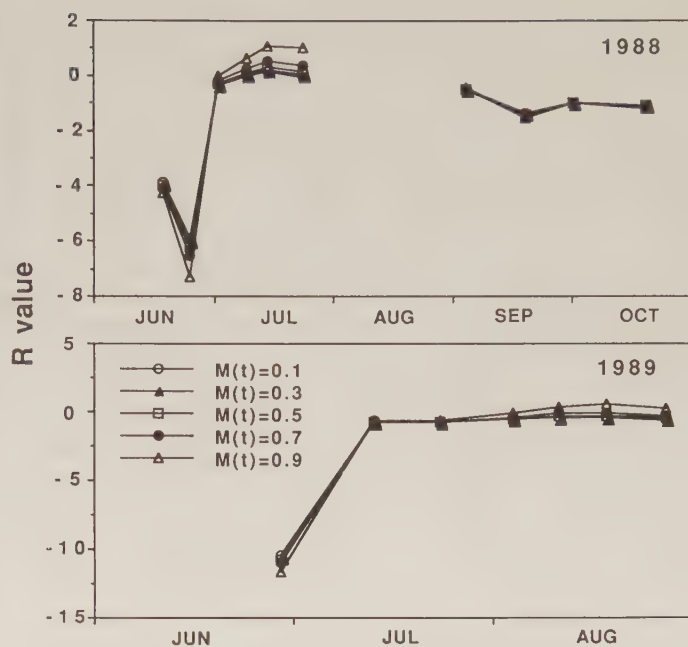


FIG. 3. Values of R , ratio of biomass change due to emigration over biomass change due to consumption by northern pike, for fish in Bolger Bog in 1988 (upper panel) and 1989 (lower panel). R is calculated for different values of $M(t)$, nonpredatory mortality rates, because $M(t)$ is difficult to estimate.

lake perimeter for all species except creek chub (Fig. 2). Creek chub showed preference for the outflow channel after the introduction of northern pike although the catch differences between the lake perimeter and the outflow channel were very small.

In 1987, the estimated biomass of prey fish assuming the absence of northern pike (without predation) was much greater than the estimated biomass assuming northern pike present plus consumption by northern pike (with predation + consumption). This was indicated by a high R value ($R > 1$) (Fig. 7, He and Kitchell 1990). The R values were much lower in 1988 and 1989 than those in 1987. In fact, R values were negative in June of both years for all $M(t)$ values and were smaller than 1.0 at almost all sampling dates in both 1988 and 1989 (Fig. 3).

Ordinations of DCA for relative abundance (percentage catch) are presented in Fig. 4. Ordinations of relative and absolute abundance were similar; therefore, only the ordination of relative abundance is presented. The first axis (DCA1) accounted for about 60% of variance, and the second axis (DCA2) accounted for about an additional 30%. Northern redbelly dace have significant negative correlations with DCA1 whereas sunfish have significant positive correlations with DCA1 (Table 2). Thus, samples with a relatively high abundance of northern redbelly dace and a relatively low abundance of sunfish will have low scores on DCA1; conversely, samples with a relatively low abundance of northern redbelly dace and relatively high abundance of sunfish will have high scores on DCA1. DCA1 accounts for the single largest proportion of variance in the data; thus, the major difference among samples is correlated with variances in abundance of northern redbelly dace and sunfish. Golden shiner is positively correlated with DCA2 ($r = 0.70$, $p < 0.05$), suggesting that the abundance of golden shiner is related to variance unexplained by DCA1.

Overlay of the secondary matrix on DCA1 indicates that DCA1 is positively correlated with day of year ($r = 0.72$, $p < 0.05$) (Table 2). Therefore, the major component of variance in the data are those calculated within-year. This is also

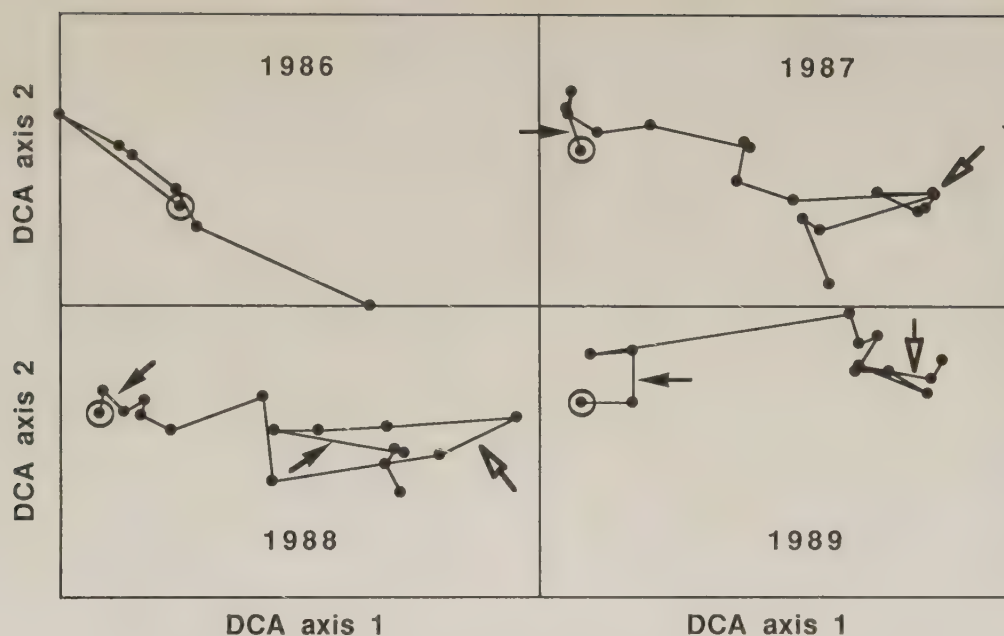


FIG. 4. DCA ordinations of relative abundances of prey species from 1986 to 1989. Scales of both axes are the same for all four panels. Lines join sample points according to the sequence of sampling. Circled points are samples from the first sampling date of each year. Open arrows indicate the dates of northern pike introduction, while solid arrows indicate the dates of northern pike removals.

TABLE 2. Correlations of DCA scores with relative species abundance and two environmental factors for Bolger Bog, 1986–89. Data used in DCA were transformed to relative abundances of prey species. All correlations are expressed as product-moment correlation (r) except for the environmental factor, year, which is expressed as Kendall's rank correlation (τ).

	Axis 1	Axis 2
Species		
Northern redbelly dace	-0.95 ^a	-0.49
Central mudminnow	0.49	-0.49
Fathead minnow	-0.60	-0.49
Brook stickleback	0.06	-0.41
Finescale dace	0.49	-0.46
Brassy minnow	-0.11	-0.23
Golden shiner	0.12	0.70 ^a
Blacknose shiner	-0.28	0.47
Sunfish	0.90 ^a	-0.50
Creek chub	0.65	-0.30
Yellow perch	0.40	0.60
Environmental factors		
Year	0.20	0.69 ^a
Day of year	0.72 ^a	-0.53
Northern pike consumption	0.35	-0.19

^aProbability of obtaining observed product-moment correlation or Kendall's rank correlation if true value is $|0.5|$ (two-tailed test) < 0.05 .

supported by the evidence that samples from the beginning of the summer for all four years appear on the same area of the ordination space (Fig. 4). Overlay of the secondary matrix on DCA2 indicates that DCA2 is correlated with year ($r = 0.69$, $p < 0.05$) (Table 2). Relating the species associations with DCA axes, it becomes clear that northern redbelly dace and sunfish are the two most important species in explaining the within-year variation. By contrast, the abundance of golden shiner is most sensitive to year-to-year variation.

Correlations between the cumulative consumption by northern pike and both DCA axes are not significant ($r = 0.35$ for DCA1, $r = -0.19$ for DCA2, $p > 0.20$) (Table 2). Cumulative consumption by northern pike is partially correlated with both day of year (correlated with DCA1) and year (correlated with DCA2). In other words, the effects of northern pike are neither orthogonal nor parallel to the ordination space defined by the DCA axes. To see effects due to northern pike predation, a multidimensional statistical test (MRPP) is needed.

The MRPP test shows that sample groups defined by years and periods of presence-absence of northern pike were significantly separated in ordination space ($p < 0.005$). This suggests that both year and presence-absence of northern pike were related to changes in the community structure of prey fish. The effects of northern pike predation also can be seen by tracing the sample trajectories on the ordination space along with the manipulation schedules of northern pike (Fig. 4). For example, although DCA1 is highly correlated with day of year, samples from the later periods of each year (September–October) do not have the highest scores on DCA1. Samples with the highest scores on DCA1 are those from late summer when northern pike were present (Fig. 4). In late August of 1987 and late July of 1988, removing northern pike from the lake caused sample points to move to the left along DCA1, suggesting that immigration of northern redbelly dace might occur in the absence of northern pike. In late August of 1988, reintroduction of northern pike caused sample points to move back to the right along DCA1.

Ordination of the same set of data using MDS shows patterns similar to those seen with DCA. Northern redbelly dace, with the highest negative correlation with MDS1 (axis 1), and sunfish, with highest positive correlation with MDS2, are still the two most important species in explaining variances in the data. The MRPP test also shows that sample groups defined by year and periods of presence-absence of northern pike are significantly separated in MDS ordination space ($p < 0.0005$). How-

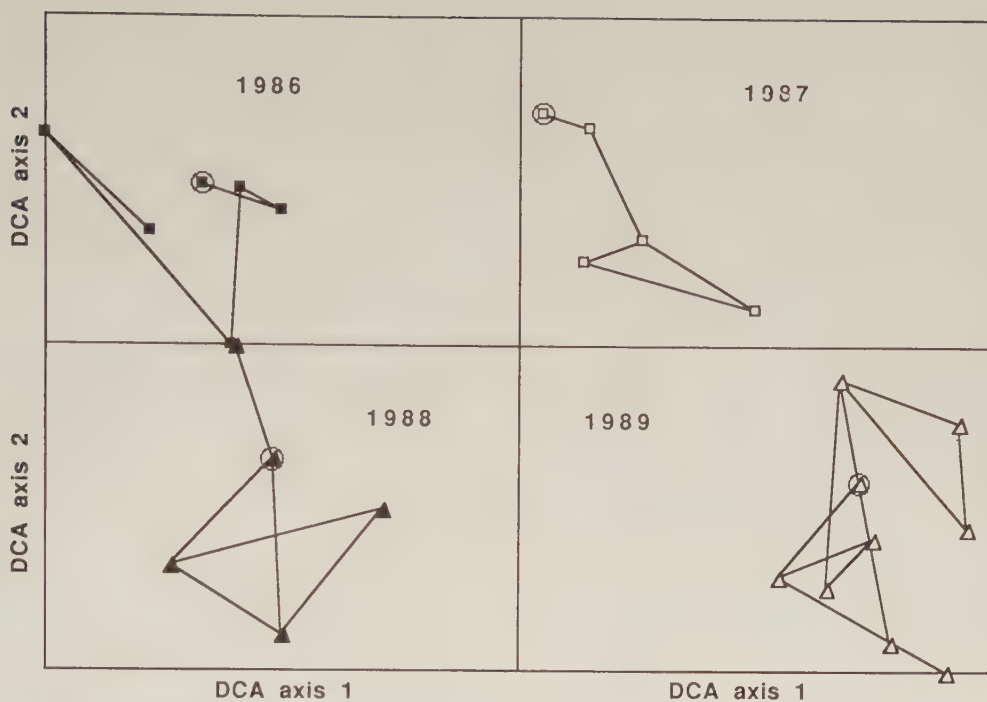


FIG. 5. DCA ordinations of presence-absence of prey species from 1986 to 1989. Details are the same as in Fig. 4.

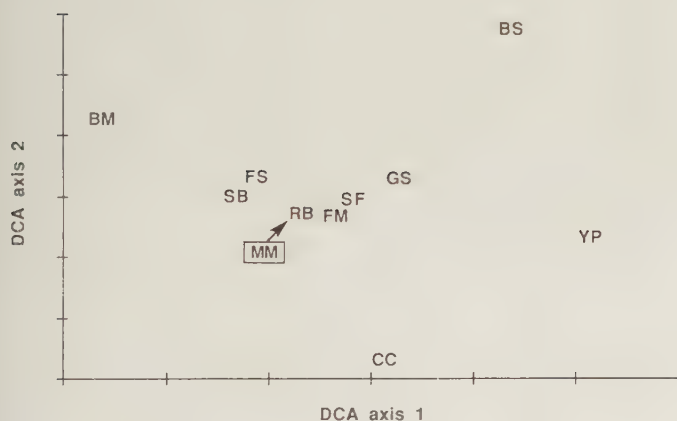


FIG. 6. DCA ordinations of presence-absence of prey species. See Table 1 for species abbreviations. MM is overlapped with RB. Note that ordination space is most determined by rare species (BM, BS, CC, and YP).

ever, MDS axes are less correlated with any of the species and environmental factors than are DCA axes. In fact, none of correlations between MDS axes and species or environmental factors is significant at a correlation coefficient of 0.5.

DCA ordination on the same data set, after presence-absence transformation, shows patterns that are very different from those of the previous analyses (Fig. 5). First, most of the variance in the data is generated by the occurrence of rare species. This can be seen in the plots of species on the DCA sample space (Fig. 6). Four relatively rare species, yellow perch, brassy minnow, blacknose shiner, and creek chub, are located on the ends of the first two DCA axes, while the other species appear to clump in the center. Second, as rare species become important, common species, such as northern redbelly dace, become less important. Therefore, the effect of northern pike predation, which had its primary effect on the abundance of common species, becomes less important. There are no significant corre-

lations between cumulative consumption of northern pike and any DCA axis ($p > 0.3$). The MRPP test also shows that sample groups defined by periods of presence-absence of northern pike were not significantly separated in ordination space ($p > 0.20$). Third, some rare species, such as yellow perch and blacknose shiner, only appeared in 1988 and 1989, causing year-to-year variance to become more important. This can be seen from the ordination graph (Fig. 5) where all sample points from 1986 have low scores on DCA1, while all sample points from 1989 have high scores on DCA1. The year-to-year variance accounts for about 58% of variance in the data. DCA1 also had significantly positive correlation with year ($r = 0.78$, $p < 0.05$). The MRPP test shows that only year is a significant factor in defining groups in ordination space ($p < 0.0005$).

The prey fish assemblage appeared unstable ($\chi^2 = 860$, $p < 0.0001$) (Table 3) across all years when the contingency table analysis was used. By contrast, in the analysis of Kendall's W , the prey fish assemblage is stable ($W = 0.41$, $p < 0.001$) (Table 3). This suggests that community stability depends on the numerical resolution of measurements used. Species absolute abundance, which was used in contingency table analysis, is much more sensitive to environmental changes than species abundance rankings, which were used in Kendall's W .

The perception of the relative importance of northern pike predation in affecting prey community structure is dependent on the level of resolution at which community structure is analyzed (Table 3). At the high and medium levels of resolution, i.e. the absolute abundance, relative abundance, and ranked abundance of prey fish, the effect of predation appears significant. But at low resolution, i.e. presence or absence, significant predation effects are not detectable. The importance of individual species to the community structure also changes at different levels of resolution. Northern redbelly dace, sunfish, and golden shiner are important species in the analysis using absolute abundance and relative abundance. No species appears

TABLE 3. Summary of analytical methods and levels of numerical resolution on evaluations of the stability of the prey fish community (upper table) and environmental effects (lower table). See Table 1 for species abbreviations.

Analytic method	Level of numerical resolution	Fish assemblage stable?			
Contingency table analysis	High	No	$(\chi^2 = 860, p < 0.001)$		
Kendall's coefficient of concordance (W)	Low	Yes			
			$(W = 0.41, p^a < 0.001)$		
		Effect			Important species
		Year	Pike	Day	
Absolute abundance/DCA	High	Yes	Yes	Yes	RB, SF, GS
Relative abundance/DCA	High	Yes	Yes	Yes	RB, SF, GS
Ranked abundance/MDS	Medium	Yes	Yes	No	None
Presence-absence/DCA	Low	Yes	No	No	BS, YP, BM, CC

^aProbability of obtaining observed Kendall's W if true value is 0.

uniquely important in the analysis using ranked abundance. Blacknose shiner, yellow perch, brassy minnow, and creek chub become important in the analysis using presence-absence of species.

Discussion

He and Kitchell (1990), based on the first 2-yr experiment in Bolger Bog, have shown the importance of indirect effects of predation on prey fishes (increase of emigration rates). Applying the same analytical methods to data collected over four sequential years, we find that the indirect effects can be less important than direct effects (consumption of prey by predator).

We suggest that lower biomass of prey fish in 1988 and 1989 than in 1987 might be the most important factor governing the relative importance of emigration to direct consumption in determining the abundance of prey fishes in Bolger Bog. There are two reasons why the relative importance of these effects would depend on the prey biomass. First, if prey fish density is not the limiting factor to feeding rate, consumption by the predator population is determined by the number of predators and the temperature of the environment (Kitchell et al. 1977; Bevelhimer et al. 1985). When prey biomass is low but not limiting, this consumption accounts for a larger proportion of the change in prey biomass than when prey biomass is high. Second, when prey fish biomass is high, competition among prey may be intense, causing prey fish to leave the lake to avoid both competition and predation. When prey fish biomass is low and competition is less intense, prey fish may risk predation to achieve higher growth or reproduction in the lake (*sensu* Gilliam and Fraser 1988). At low biomass, without the added effect of competition, the indirect effects of predation risk (e.g. emigration) are likely to be less important.

Bolger Bog is a lake that experiences winterkill and its fish assemblage is reestablished through immigration each spring; therefore, the effects of northern pike on the prey fish community have greater influence on short-term dynamics (i.e. greater within-year variance) than on long-term dynamics. Extrapolating predictions of long-term effects of northern pike predation based on the current observations of Bolger Bog could be misleading. The effect of a coexisting predator population could also be completely different from those observed in this study. However, some consistent responses were apparent based on 4 yr of data collected at the whole-lake scale. These

may provide some insight for long-term prediction. For example, when northern pike were present in all three manipulated years (1987–89), the abundance of northern redbelly dace always decreased, and the abundance of sunfish increased. The variance in abundance of these two species also corresponds to the greatest variance in the ordination analysis. Analysis of the data from Bolger Bog suggests that under strong persistent predation from northern pike, the prey fish community might shift from dominance of small-bodied cyprinids, i.e. northern redbelly dace, finescale dace, and fathead minnows, to a system dominated by larger, deep-bodied fishes, many of which have morphological defenses such as spines, i.e. sunfish, yellow perch, creek chub, and golden shiner. Rapid reduction of the small-bodied forms might allow the successful establishment and improved production of the larger species rare in the absence of predation.

These predictions are similar to those based on species-specific responses to predation (He and Kitchell 1990). This suggests that the responses of a multi-prey-fish community to predation could be predicted from the behavior and population responses of individual species. However, there are advantages in using community analysis as compared with the analysis based on individual species. First, community analysis takes advantage of the relationship between species without the assumption of independence. Second, community analysis simplifies and summarizes the data, revealing the structure in fewer key components (Gauch 1982).

Absolute and relative abundance of species are most often used in fish community analysis (Tonn and Magnuson 1982; Moyle and Vondracek 1985; Ross et al. 1985; Freeman et al. 1988), partially because most studies are conducted over relatively short periods, usually less than two generation times of the species studied. If study periods are short, absolute abundances are more likely to be sensitive response variables. Most studies also show that communities are unstable when abundances of species are used but become stable when abundance ranks or presence-absence of species are used (Yant et al. 1984; Moyle and Vondracek 1985). For this study, presence-absence of prey species is a scale too coarse to evaluate the predation effects because predation failed to eliminate species from the samples. Local extinction of species appears to require a time course longer than the length of our experiments or under greater predation intensity than we established in Bolger Bog. Absolute, relative, and ranked abundances of prey species were

all sensitive enough to detect predation effects, as we did observe shifts in species abundance.

In summary, this study demonstrates that predation by northern pike can reduce total prey biomass and change species composition in the prey fish community in Bolger Bog and that these effects can occur over a short period of a few weeks. Behavioral responses (i.e. increase of emigration) can account for a significant portion of the observed effects; however, indirect effects also seem important, but can be detected only when prey fish biomass is high. Evaluation of community persistence under predation pressure can also depend on the numerical scales of species abundances and analytic methods used. Whether these short-term responses reflect the long-term effects of piscivorous predation requires a better understanding of the compensatory response capacity of the entire community. If northern pike persisted and reproduced in Bolger Bog, responses of the prey fish community would be fundamentally different from those observed in the experiment. Under persistent pike predation the fish assemblage in Bolger Bog would shift from *Umbra*-cyprinid to centrarchid-*Esox*, two typical assemblages observed in northern Wisconsin lakes (Tonn and Magnuson 1982). Experiments designed to understand community persistence and the dynamics during the transition are needed and certainly will enhance our ability to forecast local extinctions and invasions in community development as a consequence of predator-prey interactions in fish communities.

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Taxonomic Level Sufficient for Assessing a Moderate Impact on Macroinvertebrate Communities in Puget Sound, Washington, USA¹

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Ferraro, S. P., and F. A. Cole. 1992. Taxonomic level sufficient for assessing a moderate impact on macroinvertebrate communities in Puget Sound, Washington, USA. *Can. J. Fish. Aquat. Sci.* 49: 1184–1188.

Macroinvertebrate data obtained using three sampling schemes (0.06-m² × 8-cm-deep sampling unit and 1.0- or 0.5-mm-mesh sieves, and 0.1-m² × 8-cm-deep sampling unit and 1.0-mm-mesh sieve) previously identified as optimal or near-optimal for detecting differences between a reference and a moderately impacted station when animals were identified to species were reanalyzed at the genus, family, order, and phylum level to determine the taxonomic level sufficient to detect differences between the stations with t-tests on five measures of community structure. Taxonomically sufficient levels for number of taxa were family in 1.0-mm-mesh samples and species in 0.5-mm-mesh samples. Specific identification was usually required for a Dominance, Shannon's, 1 - Simpson's, and McIntosh's Index in 1.0- and 0.5-mm-mesh samples, suggesting limits to the utility of the taxonomic sufficiency concept when using those indices to detect moderate impacts. This and a previous study indicate that one could reliably ($\alpha = 0.05$, $1 - \beta \approx 0.80$) detect moderate benthic impacts at the study site on number of taxa and five other measures of community structure with five to seven replicate 0.06-m² × 8-cm-deep, 1.0-mm-mesh samples per station and identification to family only. Taxonomic sufficiency can vary depending upon the animal size fraction sampled and the measure used.

Des données relatives aux macroinvertebrates ont été réanalysées aux niveaux du genre, de la famille, de l'ordre et du phylum, en vue de déterminer à quel niveau taxonomique minimal on doit se rendre pour détecter les différences entre les stations, à l'aide de tests t portant sur cinq mesures de la structure de la biocénose. On a obtenu ces données en utilisant trois plans d'échantillonnage (une unité d'échantillonnage de 0,06 m² × 8 cm de profondeur et un tamis dont les mailles ont 1,0 et 0,5 mm, et une unité d'échantillonnage de 0,1 m² × 8 cm de profondeur avec un tamis à mailles de 1,0 mm); ces plans avaient été auparavant évalués, par l'identification des organismes au niveau de l'espèce, comme étant les méthodes optimales ou quasi-optimales pour détecter les différences entre une station de référence et une station qui subit un impact modéré. Pour les échantillons recueillis avec un maillage de 1,0 mm, on a établi le niveau de la famille comme étant le niveau taxonomique minimal pour un certain nombre de taxons, et pour les tamis à mailles de 0,5 mm, le niveau minimal est celui de l'espèce. En règle générale, il fallait procéder à l'identification de l'espèce pour les indices de dominance, de Shannon, de 1 - Simpson et de McIntosh dans les échantillons provenant de quadrats à mailles de 1,0 et de 0,5 mm; cela signifierait qu'il y a des limites à l'utilité du concept des niveaux taxonomiques suffisants lorsque l'on se sert de ces indices pour détecter une perturbation modérée. Ces résultats, tout comme ceux d'une étude antérieure, indiquent que l'on peut détecter avec un certain degré de fiabilité ($\alpha = 0,05$, $1 - \beta = 0,80$) un impact benthique modéré sur un certain nombre de taxons du terrain à l'étude, et cinq autres mesures de la structure de la communauté au moyen de cinq à sept réplicats par station, les quadrats mesurant 0,06 m² × 8 cm de profondeur et le tamis ayant un maillage de 1,0 mm; l'identification s'arrête à la famille seulement. Le degré de suffisance taxonomique peut varier suivant la catégorie de taille des spécimens que l'on a étudiée, ainsi qu'en fonction de la mesure utilisée.

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Species-level identification may not always be necessary for pollution assessment and monitoring (Edwards et al. 1975; Hellawell 1977; Green 1979; McIntyre et al. 1984; Waterhouse and Farrell 1985; Heip et al. 1988; Warwick 1988a, 1988b; Kingston and Riddle 1989; Ferraro and Cole 1990; Gray et al. 1990). Identifying organisms to the taxonomic level necessary and sufficient to meet a study's objective(s) has been called "taxonomic sufficiency" (Ellis 1985). Taxonomic sufficiency is a pragmatic concept in which the level of identification is balanced against the need for information. Information obtained when identifying organisms to a lower

taxonomic level is considered redundant when the same inference is drawn when organisms are identified to higher taxonomic levels. The cost efficiency of macroinvertebrate studies can be greatly improved if the optimum sampling unit and sieve mesh size are used to collect organisms (Ferraro et al. 1989; Kingston and Riddle 1989) and organisms are identified only to the highest taxonomic level sufficient for the purpose of the study (McIntyre et al. 1984; Warwick 1988b).

In this study, data from macroinvertebrate samples obtained using three sampling schemes previously identified as optimal or near-optimal for detecting a moderate difference in macroinvertebrate communities at the study site when organisms were identified to species (Ferraro et al. 1989) were reanalyzed at the genus, family, order, and phylum level to determine taxonomic suf-

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iciency on five measures of community structure. A finding of sufficiency with a higher taxonomic level than species would reduce the cost of sample processing without an appreciable loss in statistical rigor and, thus, better define the optimal sampling and sample processing scheme for assessing environmental impacts. Since the ecological difference between stations was moderate (an 11.5–37.3% difference in the mean number of species and a 3.8–11.5% difference in the mean $\log_{10}$ (abundance)), depending upon the sampling scheme used (Ferraro et al. 1989)), we believed that this would be a good test of the limits of utility of the taxonomic sufficiency concept, since one would expect that more information (i.e. identification to lower taxa) may be necessary to detect moderate impacts than large impacts. In addition, by comparing taxonomic sufficiency among three sampling schemes and five measures, we were able to test the dependency of taxonomic sufficiency upon the sampling unit, sieve mesh size, and the measure used.

Materials and Methods

The field work and sample processing methods are described in Ferraro et al. (1989). Basically, replicate macrobenthic samples were collected using a 0.06-m² box corer and a 0.1-m² van Veen grab at a reference station and a moderately impacted station in the vicinity of a fuel depot in the Everett Harbor area of Puget Sound. The stations were similar in most respects (water depth, sediment grain size, and organic carbon content, etc.), but concentrations of high molecular weight aromatic hydrocarbons in the sediments were between one and two orders of magnitude higher at the impacted station (PTI Environmental Services and Tetra Tech 1988). Samples were sieved through 1.0- and 0.5-mm-mesh stacked sieves to isolate two animal size fractions: ≥ 1.0 mm (1.0-mm-mesh samples) and ≥ 0.5 mm (0.5-mm-mesh samples). The material retained on each sieve was fixed with 10% buffered formalin in seawater. Samples were transferred to 70% ethanol, stained with rose bengal, and sorted under a dissecting microscope (6–16 $\times$). Specimens were identified to the lowest possible taxa, usually species, and counted.

Because Foraminifera are difficult to sort and are rarely identified to species in environmental studies, we excluded them from this study. We also confined this study to data from samples collected using the overall optimal sampling scheme at the study site (0.06-m² $\times$ 8-cm-deep sampling unit and 1.0-mm sieve mesh size) and to two near-optimal schemes (0.1-m² $\times$ 8-cm-deep sampling unit and 1.0-mm sieve mesh size, and 0.06-m² $\times$ 8-cm-deep sampling unit and 0.5-mm sieve mesh size) (Ferraro et al. 1989).

Data were grouped by taxa (species, genus, family, order, phylum), and for each sampling scheme, taxonomic level, and station, we calculated five measures of community structure: (1) number of taxa, (2) a Dominance Index equal to the minimum number (or fraction) of taxa whose combined abundance was equal to 75% of the individuals (Swartz et al. 1986), (3) Shannon's Index, H' (Shannon and Weaver 1964), (4) the complement of Simpson's Index (Simpson 1949), and (5) McIntosh's Index (McIntosh 1967, expression 4). Means and variances for measures 1 and 2 were calculated in the standard way. Jackknife statistics were calculated for measures 3–5 as described in Ferraro et al. (1989) and Ferraro and Cole (1990). Normality was tested by the W statistic (Shapiro and Wilk 1965) and homogeneity of variance by $F_{\max}$ (Sokal and Rohlf 1981). The significance level was $p < 0.05$ in all tests.

Statistical power analyses for a two-tailed t -test (Cohen 1977; Zar 1984; Krebs 1989) were conducted using the computer pro-

gram POWER (Computing Center, University of Texas, Austin, TX 78712) and confirmed using DESIGN-POWER (Bavry 1987). Alpha was set to 0.05. The error term was the mean square within stations, i.e. the average variance at the two stations. Our approach was to first determine the number of replicate samples per station, n^* , needed to obtain an estimated statistical power ($1 - \hat{\beta}$) as close as possible to 0.80 for the mean difference observed between stations when animals were identified to species. The value of 0.80 was chosen as the reference point from which to observe $1 - \hat{\beta}$ as a function of the taxonomic level of identification because it is a high value from which we could observe marked increases and decreases in $1 - \hat{\beta}$. Using n^* , we then determined $1 - \hat{\beta}$ for the mean difference observed between stations when animals were identified to genus, family, order, and phylum. Our criterion for taxonomic sufficiency was that $1 - \hat{\beta}$ was not appreciably different ($\Delta \leq 0.10$) than that when animals were identified to species and progressively higher taxa. For example, if $1 - \hat{\beta} = 0.80$ for species, a higher taxonomic level was deemed sufficient if and only if its $1 - \hat{\beta}$ and the $1 - \hat{\beta}$ of all lower taxonomic levels were ≥ 0.70 and ≤ 0.90 . We discounted results when $n^* > 20$, since the taxonomic sufficiency of such insensitive measures is irrelevant. Other reasonable critical values for Δ and n^* would not have changed our results appreciably.

Results

Summary statistics for measures 1–5 when animals were identified to species were presented by Ferraro et al. (1989, table 3). Summary statistics when animals were identified to genus, family, order, and phylum are available from the authors upon request. Ninety-six percent (144 of 150) of the normality tests and 95% (71 of 75) of the variance tests were not statistically significant. Where violations of the parametric assumptions did occur, they probably had only a minor effect on our estimates of statistical power (Glass et al. 1972; Ferraro and Cole 1990).

Family-level identification was sufficient for number of taxa in 1.0-mm-mesh samples (Fig. 1a and 1b), but species-level identification was needed in the 0.5-mm-mesh samples (Fig. 1c) (Table 1). Species-level identification was usually needed for the Dominance, Shannon's, $1 - \text{Simpson's}$, and McIntosh's indices in 1.0- and 0.5-mm-mesh samples (Fig. 1; Table 1). The similarity of some of the $1 - \hat{\beta}$ response curves is not surprising considering the mathematical similarities of some of the indices (Heltshe and Bitz 1979). We do not attribute any biological significance to the spikes in the $1 - \hat{\beta}$ response curves at the order and phylum level of identification on some measures. They may be simply due to happenstance. As a practical matter, they are irrelevant to the determination of taxonomic sufficiency in this study, since they are preceded by marked decreases in $1 - \hat{\beta}$ at the genus and family level of identification.

Sampling unit area (0.06 versus 0.1 m²) appeared to have little effect on taxonomic sufficiency (Table 1). The Dominance Index was very insensitive using the 0.06- and 0.1-m², 1.0-mm-mesh sampling schemes as indicated by the large sample sizes ($n^* = 40$ and 518, respectively) needed to reliably ($\alpha = 0.05$, $1 - \hat{\beta} \approx 0.80$) detect a significant difference between stations (Table 1). Shannon's Index was also very insensitive in the 0.1-m², 1.0-mm-mesh samples ($n^* = 145$; Table 1).

Discussion

This study expands upon Ferraro et al.'s (1989) research to determine the optimum macrobenthic sampling scheme (sampling unit, sieve mesh size, and n) by the addition of taxonomic level of identification as a sample processing variable. Ferraro et al.'s (1989) power-cost efficiency (PCE) approach is not amenable to the taxonomic sufficiency problem because taxonomic sufficiency requires comparable statistical rigor when analyses are conducted on data grouped to all lower taxonomic levels. Even omitting this requirement, the PCE approach would not be well suited for determining taxonomic sufficiency, since the cost of identification to different taxonomic levels is difficult to estimate accurately and can vary considerably depending upon the taxonomic expertise of the persons performing the identifications and the taxonomic composition of the samples. When seeking the optimum macrobenthic sampling and sample processing scheme, we advise first determining the optimum (or near optimum) sampling unit, sieve mesh size, and n by the PCE method and then taxonomic sufficiency by the method in this paper.

To determine the optimum macrobenthic sampling and sample processing scheme for our study area, it is necessary to consider the results of this study and those of Ferraro et al. (1989). Ferraro et al. (1989) tested six other measures of community structure, five (total biomass, biomass by major taxa (Polychaeta, Mollusca, Crustacea), numerical abundance) of which did not require identification to taxa lower than order. The sixth measure, the Infaunal Index (Word 1990), is based on the relative abundance of 163 taxa (species through phylum) in six groups. All of the five measures requiring order or higher taxonomic identification were as sensitive or more sensitive than the measures tested in this study under the $0.06\text{-m}^2 \times 8\text{-cm-deep}$, 1.0-mm-mesh sampling scheme ($n^* = 5\text{--}7$). One could, therefore, reduce the sample processing cost and still reliably detect moderate or larger deviations from reference conditions at the study site on number of taxa (probably the most universally accepted measure of community structure) and five other measures with five to seven replicate $0.06\text{-m}^2 \times 8\text{-cm-deep}$, 1.0-mm-mesh samples per station and identification of animals to family only.

With one exception ($1 - \text{Simpson's Index}$ under the $0.06\text{-m}^2 \times 8\text{-cm-deep}$, 0.5-mm-mesh sampling scheme), taxonomic sufficiency remained at the species level for the dominance and three diversity indices (Table 1). These results suggest that the taxonomic sufficiency concept may have limited utility when using those indices to detect moderate impacts on benthic communities. Gray (1981) faulted diversity indices for their low sensitivity to moderate pollution effects, but others (e.g. see references in Magurran (1988)) have found diversity indices to be useful measures of stress. Species dominance and species diversity indices were sensitive measures of community differences in our 0.06-m^2 , 0.5-mm-mesh samples, but sensitivity tended to decrease as the level of taxonomic identification increased (Fig. 1c). When the taxonomically sufficient level is species, obviously the limits of utility of the taxonomic sufficiency concept have been reached.

The difference in sensitivity of the dominance and diversity indices between the 0.06-m^2 , $\geq 1.0\text{-mm}$ and $\geq 0.5\text{-mm}$ samples was due to a greater number of species, a more even distribution of individuals among species, and a lower variance of the indices in the $\geq 0.5\text{-mm}$ samples. Low sensitivity of diversity indices in some macrobenthic pollution studies could be due in part to the animal size fraction investigated. Taxa with smaller

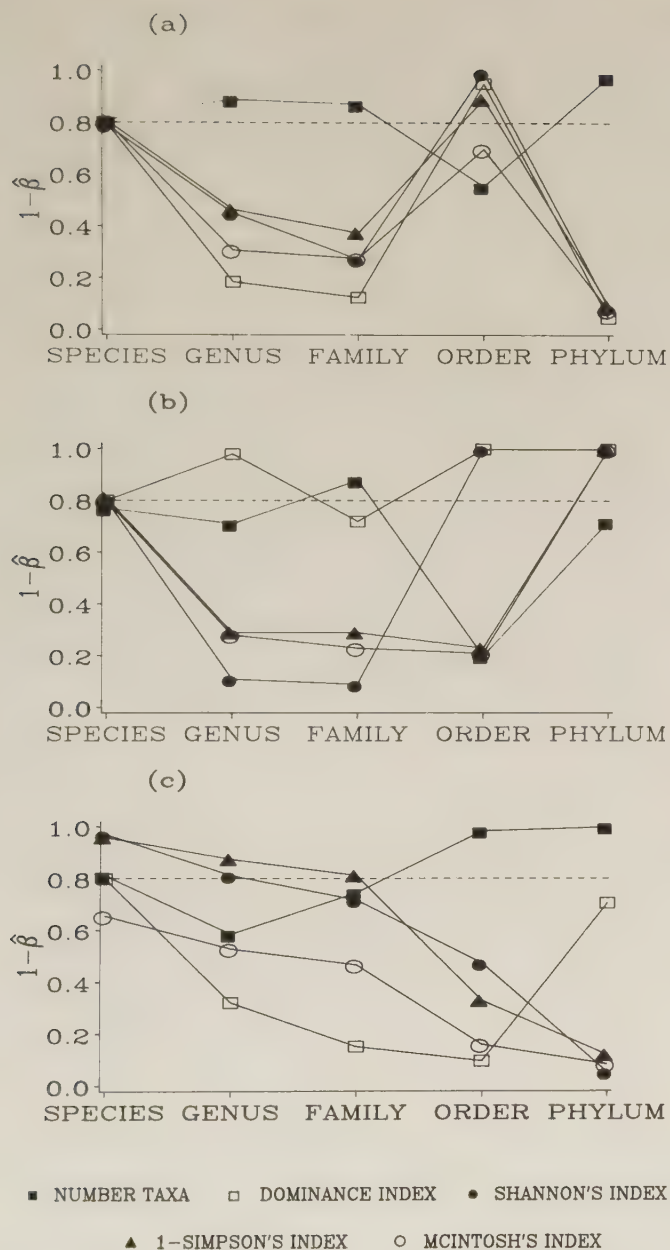


FIG. 1. Estimated statistical power ($1 - \hat{\beta}$) as a function of the level of taxonomic identification for the (a) $0.06\text{-m}^2 \times 8\text{-cm-deep}$, 1.0-mm sieve mesh size sampling scheme, (b) $0.1\text{-m}^2 \times 8\text{-cm-deep}$, 1.0-mm sieve mesh size sampling scheme, and (c) $0.06\text{-m}^2 \times 8\text{-cm-deep}$, 0.5-mm sieve mesh size sampling scheme. Alpha = 0.05. The numbers of replicate samples per station (n^*) used to estimate $1 - \hat{\beta}$ are given in Table 1.

The sieve mesh size used to sort animals had a marked effect on the statistical power and taxonomic sufficiency of number of taxa; n^* increased from 5 to 15 (i.e. for a given sample size the $1 - \hat{\beta}$ decreased) and taxonomic sufficiency went from family to species when the 0.5- to 1.0-mm animal size fraction was included in the analyses of the 0.06-m^2 samples (Table 1). There was little or no effect of sieve mesh size on taxonomic sufficiency of the other four measures. Statistical power, however, was much higher for the $\geq 0.5\text{-mm}$ than the $\geq 1.0\text{-mm}$ samples; n^* 's for the four indices were 7–40 for the 0.06-m^2 , 1.0-mm-mesh samples and only 2–5 for the 0.06-m^2 , 0.5-mm-mesh samples (Table 1).

TABLE 1. Highest taxonomic level needed to reliably ($1 - \hat{\beta} \approx 0.80$) detect a significant ($\alpha = 0.05$) difference between a reference and a moderately impacted station in Puget Sound, Washington, for three sampling schemes and five measures of community structure. The number of replicate samples required per station (n^*) is given in parentheses.

Measure	0.06 m ² , ≥ 1.0 mm	0.1 m ² , ≥ 1.0 mm	0.06 m ² , ≥ 0.5 mm
Number taxa	Family (5)	Family (8)	Species (15)
Dominance Index	Species (40) ^a	Species (518) ^a	Species (5)
Shannon's Index	Species (19)	Species (145) ^a	Species (3)
1 - Simpson's Index	Species (11)	Species (14)	Genus (3)
McIntosh's Index	Species (7)	Species (9)	Species (2)

^aThese results were discounted, since $n^* \geq 20$.

animals tend to dominate under polluted conditions (Pearson and Rosenberg 1978), and therefore, differences in diversity between reference and polluted sites may be more evident when smaller macrofauna are included in the samples. But if the smaller animals present are primarily ephemeral, patchily distributed juveniles of larger macrobenthic species, the use of a smaller mesh sieve may be counterproductive in a pollution impact study (Hartley 1982).

Our general finding is that the taxonomic sufficiency to detect pollution impacts on macrobenthic communities can vary depending upon the animal size fraction sampled and the measure used (Table 1). We are unaware of any other study of taxonomic sufficiency on the ≥ 1.0 - and ≥ 0.5 -mm animal size fractions from the same macrobenthic samples. Different responses to anthropogenic disturbances, however, have been reported for macrobenthic and meiobenthic communities (Austen et al. 1989; Warwick et al. 1990). Apparently, some stressors can act more on one animal size component of benthic communities than another, and the sensitivity of diversity indices to changes in macrobenthic communities can be influenced by the level of taxonomic identification (also see Wu 1982).

Obviously, a greater prevalence of monotypic genera (or monotypic higher taxa) will tend to increase the probability of taxonomic sufficiency at higher taxonomic levels than species. Also, if members of the same genus, family, etc., are similar in their response to pollution, little relevant information may be lost by identification to higher taxa. Resh and Unzicker (1975) noted a wide range in the pollution tolerances of freshwater macroinvertebrate congeners and concluded that species-level identification was essential for water quality monitoring. Many marine macroinvertebrate congeners, however, appear to have similar tolerances to organic enrichment and pollution (Pearson and Rosenberg 1978; Word 1978, 1990). Grouping data to higher taxa tends to reduce the natural variability of some community structure measures, thereby potentially improving the statistical power to discriminate reference from impacted sites using standard univariate tests (Ferraro and Cole 1990).

Warwick's (1988a, 1988b) explanation for the sufficiency of higher taxonomic levels in pollution studies is that "nuisance" variables (e.g. water depth and sediment granulometry) usually influence community structure at a lower taxonomic level than pollution, since species have had much longer to adapt to these variables than pollution. At the species level there may be confounding effects of nuisance variables and pollution on benthic communities at polluted sites whereas at higher taxonomic levels the pollution effect will tend to dominate.

Ferraro and Cole (1990) hypothesized that the sufficiency of higher taxonomic levels may be a consequence of the hierarchical structure of biological responses to stress (Pearson and

Rosenberg 1978). As a toxic stress increases, the adaptability of first the individual, and then the species, genus, family, etc., is exceeded. Consequently, impacts resulting from increasing stress are manifest at higher and higher taxonomic levels. A slight toxic stress which eliminates only species X, for example, would only be detectable if organisms were identified to species whereas a greater stress which eliminates species X and all its congeners would be detectable if organisms were only identified to genus. The hierarchic-response-to-stress hypothesis does not explain the sufficiency of higher taxonomic levels when stressors increase community species richness.

Whatever the reason(s), accumulating evidence (Heip et al. 1988; Warwick 1988a, 1988b; Ferraro and Cole 1990; Gray et al. 1990, this study) suggests that family or higher levels of taxonomic identification may often be sufficient for detecting pollution impacts on marine benthic communities. These findings should stimulate further research on taxonomic sufficiency including retrospective analyses of data from past research and monitoring programs. Since the cost savings can be considerable, we advise conducting a preliminary study to determine taxonomic sufficiency for large studies. Taxonomic sufficiency should be determined as early as possible in long-term studies, since costs can be reduced by subsequently identifying organisms only to the sufficient taxon. As information accumulates on the taxonomic sufficiency for different habitats and different amounts and kinds of pollution, it may be possible to predict with reasonable accuracy when and where identification to higher taxonomic levels will be sufficient for detecting pollution impacts.

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Osmoregulatory Failure and Death of First-Year Largemouth Bass (*Micropterus salmoides*) Exposed to Low pH and Elevated Aluminum, at Low Temperature in Soft Water

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Young-of-the-year largemouth bass (*Micropterus salmoides*) were exposed to pH levels from 8.0 to 4.5 in two water types, 1.5 and 13.4 mg Ca/L. Exposures were conducted at 3.8°C for 113 d, followed by 14 d of increasing temperature to 18°C. Two treatments in the softer water, one each at pH 5.0 and 4.5, had Al added to attain 30 µg Al/L; all other treatments were at approximately 5 µg Al/L. The condition factor of fish in all treatment groups declined with exposure time at 3.8°C. Fish in the 13.4 mg Ca/L water maintained osmotic homeostasis through pH 5.0. In the 1.5 mg Ca/L water, osmotic homeostasis was lost at pH 4.5 and at pH 5.0 when Al was added. Mortalities were most prevalent when exposed in the 1.5 mg Ca/L water with added Al. The probability of survival was directly correlated with blood osmolality; no correlation was found between survival probability and condition factor. A rise in blood osmolality occurred among fish from most exposure groups when the temperature was increased to 18°C. When fish from these chronic treatments were challenged at pH 3.8, they had shorter survival times in the softer water and after longer preexposures.

On a exposé de jeunes achigans à grande bouche de l'année (*Micropterus salmoides*) à des pH variant entre 8,0 et 4,5, l'eau contenant, dans un cas, 1,5 mg Ca/L, et dans l'autre, 13,4 mg Ca/L. Cette expérience s'est déroulée à une température de 3,8 °C pendant 113 jours, puis, pendant 14 jours, on a augmenté progressivement la température jusqu'à 18 °C. Dans deux des expériences effectuées en eau douce, l'une à un pH de 5,0, et l'autre à 4,5, on a augmenté la teneur en Al jusqu'à 30 µg Al/L; toutes les autres expériences ont été effectuées à des concentrations d'environ 5 µg Al/L. Pour tous les groupes exposés, le coefficient de condition a diminué en fonction de la durée d'exposition à 3,8°C. Dans l'eau contenant 13,4 mg Ca/L et dont le pH était de 5,0, les poissons conservaient leur homéostasie osmotique. Cependant, dans l'eau contenant 1,5 mg Ca/L, les poissons perdaient leur homéostasie osmotique à un pH de 4,5 et un pH de 5,0 si l'on y ajoutait de l'Al. On a relevé le plus grand nombre de mortalités lorsque l'eau contenait 1,5 mg Ca/L en plus de l'Al. La probabilité de survie était directement proportionnelle à l'osmolalité sanguine; on n'a pas observé de corrélation entre la probabilité de survie et le coefficient de condition. Une élévation de température à 18 °C a engendré une hausse de l'osmolalité sanguine chez les poissons de la plupart des groupes exposés. Chez les poissons soumis à ces expériences de toxicité chronique et qui étaient exposés à un pH de 3,8, on enregistrait un raccourcissement de la durée de survie, surtout si l'exposition préalable avait été plus longue.

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Effects of environmental acidification on freshwater fishes were evaluated through parallel-concurrent laboratory, in situ, and whole-ecosystem studies (Swenson et al. 1989; Watras and Frost 1989; Eaton et al., (U.S. EPA), Environmental Research Laboratory-Duluth, Duluth, MN, unpubl. data). The ecosystem studies revealed that recruitment failure of largemouth bass (*Micropterus salmoides*) in Little Rock Lake occurred at a pH higher than predicted from the in situ or laboratory embryo-larval studies. The lake is located in north-central Wisconsin and has two nearly equally sized basins separated by a narrows. The Little Rock Lake acidification study progressed from an initial 1.5 yr of study of its intact biological and physical characteristics, followed by installation of a plastic sea curtain across the narrows, and gradual acidification of one of its basins by addition of sulfuric acid. Acid-

ification continued at 0.5 pH increments and at 2-yr intervals, pH 5.6 (1985–86), pH 5.1 (1987–88), and pH 4.7 (1989–90), when acid additions were halted and the pH was allowed to begin recovery by natural processes. The lake had four major fish species: largemouth bass, rock bass (*Ambloplites rupestris*), black crappie (*Pomoxis nigromaculatus*), and yellow perch (*Perca flavescens*). This paper deals only with the largemouth bass, although it is believed that there are considerable implications for other warmwater fishes.

Because it has been well established that it is the embryo-larval period of a fish's life that is the most sensitive to environmental acidification (Craig and Baksi 1977; Fivelstad and Leivestad 1984; McCormick et al. 1989a), these stages were chosen for in situ and laboratory studies to predict the effects of environmental acidification on the fishes of Little Rock Lake.

The results of these parallel embryo-larval studies were in close agreement; the laboratory, in situ, and whole-ecosystem observations all found hatching and survival at pH through 5.0–5.1 and that all larvae died within a few days of hatching, when exposed to pH 4.5–4.7. However, no surviving age 1 largemouth bass were found in the acid treatment basin after the 1987–88 winter, while there was good survival in the reference (nonacid treated) basin (Eaton et al., (U.S. EPA), Environmental Research Laboratory-Duluth, Duluth, MN, unpubl. data). This young-of-the-year (YOY) largemouth bass mortality during their first winter in the lake at pH 5.1 was not predicted from the embryo-larval studies. Although toxicologically the embryos and larvae encompass the most acid-sensitive life stages, overwintering first-year juveniles were more vulnerable to environmental acidification in Little Rock Lake. The cause of this discrepancy is the subject of the work reported in this paper.

In attempting to understand what might be involved in these apparently conflicting observations, we decided to test three hypotheses: (1) that the responses of juvenile largemouth bass to environmental acidification at overwintering temperatures ($\sim 4^{\circ}\text{C}$) would be different than if exposed at temperatures extant during the spawning and growing seasons, (2) that water quality factors associated with environmental acidification, particularly mobilized Ca and Al, influence the responses of juvenile largemouth bass to a given pH in nature, and (3) that prolonged exposure of juvenile largemouth bass to winter temperatures and at different pH and Al concentrations will alter their acid sensitivity.

Shuter et al. (1980) provided evidence that the northern limit of distribution of smallmouth bass (*Micropterus dolomieu* (previously *dolomieu*)) was determined by its success or failure to survive the first winter and that survival was size dependent (Shuter and Post 1990). With this knowledge, the overwintering mortalities of Little Rock Lake's YOY largemouth bass was believed due either to more rapid consumption of stored resources while living in an acidified environment (Lee et al. 1983; Shuter et al. 1989; Leino et al. 1990) or an increased pH sensitivity caused by the prolonged overwintering fast (Ivlev 1961; Kwain et al. 1984). In attempting to answer these questions, we exposed YOY largemouth bass to conditions simulating those of overwintering in Little Rock Lake with a 14-d period of rising temperature provided at the end to simulate spring. The pH levels chosen corresponded to those planned for the acidification of the acid-treated basin, pH 5.5, 5.0, and 4.5 (Watras and Frost 1989). Calcium concentrations were regulated to simulate that of Little Rock Lake (1.2 mg Ca/L at pH 5.1; Mach and Brezonik 1989), and also to 13.4 mg Ca/L, to evaluate the effect of Ca concentration on pH effects. Aluminum additions to the two lowest pH levels, in the softer water, simulated conditions in the acidified basin during the winter of 1987–88, 37 μg soluble Al/L (Mach 1992).

Before proceeding, a number of additional factors governing the effects of environmental acidification on freshwater fishes must be affirmed, as they are the foundations to understanding the responses observed: (1) recruitment failure is most often the first population level effect observed (Beamish 1976; Haines 1981; Schindler et al. 1985), (2) effects of environmental acidification are often due to hydrogen ions working in concert with metals mobilized by low pH (Schindler et al. 1980; Baker and Schofield 1982; Hutchinson and Sprague 1986), particularly Al (Schofield and Trojnar 1980; Driscoll et al. 1980; Baker and Schofield 1982; Skogheim et al. 1984), (3) Ca availability influences the toxicity of a given hydrogen ion concentration

(Wright and Snekvik 1978; Nelson 1982; Brown 1983; McDonald 1983; Freda and McDonald 1988; Shuter and Ihssen 1991), (4) cold inhibits feeding, particularly in warmwater fishes (Johnson and Charlton 1960), (5) larger individuals of a species live longer under the same acid stress than smaller ones (Potts 1954; Muir 1969; Henderson et al. 1988; Shuter and Ihssen 1991), and (6) ionoregulatory disturbances are the cause of death after chronic low-pH exposure (Wood 1989).

Methods and Materials

Treatments and Chronic Exposure System

Juvenile largemouth bass were exposed to a series of pH and Al treatments using two pulsed continuous-flow diluters modified from Benoit et al. (1982). One of these delivered soft Lake Superior water (13.4 mg Ca/L) to fish exposure tanks, while the other delivered reverse osmosis deionized water mixed with Lake Superior water to provide a very soft water (1.5 mg Ca/L) similar to that of Little Rock Lake. Water from these diluters flowed at fixed head through capillary tubes which delivered fixed volumes to second-level mixing, degassing, and distribution troughs enroute to the fish exposure tanks. To achieve the desired pH in the exposure tanks, dilute sulfuric acid (reagent grade) was added by Mariotte stock bottle (Engstrom-Heg 1971). When Al was added, it was introduced to the acid mixing troughs as aluminum chloride ($\text{Al}(\text{Cl})_3 \cdot 6\text{H}_2\text{O}$) (Kodak reagent grade) from a separate Mariotte bottle.

Each mixing chamber of the diluter systems was aerated to remove any free CO_2 that might have been liberated by acidification. Additional aeration in the exposure tanks insured homogeneity of temperature, pH, and Al throughout each tank. The exposure tanks were 14-L glass aquaria which received approximately 100 mL/min of the respective treatment waters. The two diluter systems provided an array of exposure conditions at two Ca levels: one with 13.4 mg Ca/L water and three acid levels, pH 5.5, 5.0, and 4.5 with a control at 8.0, and the other with 1.5 mg Ca/L water at these same acid levels with a control at pH 7.0 (Table 1). Aluminum was added to a second set of the two lowest pH treatments (pH 4.5 and 5.0) in the 1.5 mg Ca/L water to attain 30 μg monomeric Al/L (Table 1). Aluminum treatments in the text are identified as "without added Al" (5 μg Al/L) or "with added Al (30 μg Al/L)" and are as nominal monomeric Al. All treatments were repeated once, duplicates A and B.

The pH was monitored at 10-min intervals using strip chart recorders. Reported exposure pH's were derived from samples drawn directly from the exposure tanks once or twice daily and measured with a temperature-compensated Beckman Φ 70 pH meter, using a Ross electrode. Aluminum samples from each exposure tank were collected once weekly and analyzed using a Perkin-Elmer model 5000 furnace atomic absorption spectrophotometry (U.S. EPA 1983). Monomeric Al determinations were made by the colorimetric catechol violet method (LaZerte et al. 1988) without dialysis or ion-exchange (Table 1). Conductivity was also measured daily in each tank as a surrogate for Ca, which was later confirmed by weekly atomic absorption spectrophotometry analysis of samples from all exposure tanks (U.S. EPA 1983) (Table 1).

Temperature was regulated by delivery of thermostatically controlled cold water to the exposure tanks and immersion of these tanks in two temperature-controlled chilled water baths. Temperature in the exposure tanks was continuously monitored by a strip chart recorder whose values were calibrated daily by

TABLE 1. Measured mean ($\pm$ SE) pH, Ca, Al, alkalinity, conductivity, and sulfate during the largemouth bass overwintering exposure.

Exposure conditions:										
Ca (mg/L)										
13.4							1.5			
							5.0		4.5	
pH (nominal)	8.0	5.5	5.0	4.5	7.0	5.5				
Al (μ g/L) (nominal)	5	5	5	5	5	5	5	30	5	30
pH ($\pm$ 1 SD) ^a	7.9 (7.8–7.9)	5.5 (5.3–6.2)	4.9 (4.8–5.2)	4.4 (4.3–4.6)	6.9 (6.9–7.0)	5.5 (5.4–5.6)	5.0 (4.9–5.1)	5.0 (4.9–5.1)	4.5 (4.5–4.6)	4.5 (4.5–4.6)
Total Al (μ g/L)	32.8 $\pm$ 5.5	33.4 $\pm$ 5.5	33.4 $\pm$ 5.7	36.5 $\pm$ 6.1	5.37 $\pm$ 0.5	4.98 $\pm$ 1.0	5.18 $\pm$ 1.0	53.9 $\pm$ 1.3	4.35 $\pm$ 0.5	54.3 $\pm$ 1.0
Monomeric Al (μ g/L) ^b	7.1 $\pm$ 0.4	7.5 $\pm$ 0.6	6.1 $\pm$ 0.4	8.6 $\pm$ 0.7	5.3 $\pm$ 0.2	5.1 $\pm$ 0.1	5.4 $\pm$ 0.3	29.2 $\pm$ 0.9	5.4 $\pm$ 0.2	31.6 $\pm$ 1.1
Alkalinity (mg CaCO ₃ /L)	40.6 $\pm$ 0.4	0.6 $\pm$ 0.2	–0.7 $\pm$ 0.4	–2.3 $\pm$ 0.1	4.8 $\pm$ 0.1	–0.2 $\pm$ 0.1	–0.5 $\pm$ 0.1	–0.6 $\pm$ 0.1	–1.9 $\pm$ 0.1	–1.7 $\pm$ 0.2
Conductivity (μ S/cm)	99 $\pm$ 0.1	120 $\pm$ 0.2	124 $\pm$ 0.2	134 $\pm$ 0.3	13.6 $\pm$ 0.1	18.3 $\pm$ 0.1	19.4 $\pm$ 0.1	19.7 $\pm$ 0.1	27 $\pm$ 0.1	27 $\pm$ 0.1
SO ₄ (mg/L) ^c	3.4	40.9	44.0	43.3	0.4	5.4	6.0	6.2	7.0	7.2

^aReported at $\pm$ 1 SD because the variance is not symmetrical around the mean on a logarithmic scale.^bDue to the low total organic carbon (<2 mg/L), it can be inferred that the Al reported as monomeric Al (LaZerte et al. 1988) is inorganic monomeric Al.^cMean of two samples only; one taken at the beginning and the other near the end of the exposures.

readings taken in each exposure tank. The experiment-wide mean temperature through the first 113 d of exposure was 3.8 ± 0.1 (SE) $^{\circ}$ C. Between the 113 and 127 d the temperature was raised 2° C every other day to 18° C to simulate spring warming. Photoperiod was adjusted weekly to that of Duluth, MN (mid-January to mid-May 1989), with a 30-min period of brightening and dimming each morning and evening respectively to simulate dawn and dusk.

Free CO₂ was measured biweekly in all tanks and was always less than 4.4 mg/L, the detection limit of the Orion specification electrode used. Alkalinity was determined twice during the exposures by Gran's plot titration (Stumm and Morgan 1981). The soft water alkalinity was 40.6 ± 0.4 mg/L and the very soft water 4.8 ± 0.1 mg/L as CaCO₃ before addition of acid. Alkalinity became progressively lower at each lower pH (Table 1). Dissolved O₂ was measured weekly with an oxygen meter and averaged 11.9 mg/L with no observed values less than 10.9 mg/L. Total water hardness was determined weekly in all tanks by the EDTA titrimetric method (U.S. EPA 1983) and was 47.6 ± 0.1 mg/L in the soft water and 4.6 ± 0.1 mg/L in the very soft water. Other characteristics (mean $\pm$ SE) of the exposure waters were as follows in respective order soft water and then very soft water: total organic C, both ≤ 2 mg/L; Mg, 2.42 ± 0.02 and 0.31 ± 0.01 mg/L; Na, 2.10 ± 0.04 and 0.37 ± 0.01 mg/L; K, 0.42 ± 0.03 and 0.08 ± 0.01 mg/L (U.S. EPA 1983); nitrate, 0.3 ± 0.1 and 0.1 ± 0.1 mg/L; Cl, $<1.7 \pm 0.2$ and 0.7 ± 0.3 mg/L. Sulfate ranged from 3.4 mg/L at pH 8.0 to 43.3 mg/L at pH 4.5 in the soft water and from 0.4 mg/L at pH 7.0 to 7.2 mg/L in the very soft water at pH 4.5. Fluoride was below detection limits of the methods used (0.05 mg/L). All anions were measured with a Dionex model 12 ion chromatograph (U.S. EPA 1984). These variables were similar from treatment to treatment except for sulfate, which increased due to addition of H₂SO₄ as pH was lowered (Table 1).

The test fish, YOY largemouth bass, were supplied by the U.S. Fish and Wildlife Service Hatchery at Genoa, WI. Before experimentation the fish received a continuous flow of Lake Superior water at seasonal temperatures (midsummer through mid-January) and were being held at 4 – 5° C when the exposure began. Distribution of 60 fish to each exposure tank was in a stratified random order, with each tank receiving five fish before the next group of five was added. The mean individual fish

weight at distribution was 3.38 (2.51–4.53) g; mean condition factor was 1.20. Condition factor (Carlander 1969) is an index of a fish's general well being and is equal to 10^5 (mean weight, g/total length, mm)³. The fish were acclimated for 14 d to 3.8° C water and the respective treatment dilution water Ca concentration before the pH and Al treatments were begun. During holding and acclimation the fish received, and fed readily, on thawed adult brine shrimp (*Artemia* sp.). During testing, the fish were fed brine shrimp, rinsed in distilled water, twice daily except on weekends and holidays when one feeding per day was provided. Feeding rate was estimated subjectively and uneaten food was removed after each feeding. Food was provided because we had no evidence that food would not be available or utilized by fish during the winter under natural conditions (Brandt and Flickinger 1987). Shelters were not provided because they were considered unnecessary for these fish experiencing winter torpor (Johnson and Charlton 1960).

To follow the temporal effects of the treatments, three to five fish were removed from each tank at 42, 84, 113, and 127 d of exposure to monitor condition factors and blood osmolality. Baseline blood osmolality values were obtained from 10 fish both prior to and after the 14-d acclimation period. Blood was obtained by severing the caudal peduncle, drawing 7 μ L of blood into an Eppendorf-type pipette, and osmolality measured using a Wescor vapor pressure osmometer. Lengths (0.1 mm total length (TL)) and blotted dry wet weights (0.01 mg) were also determined for each fish at each sampling. These values were used to calculate condition factors (Fig. 1A).

Median Time to Death (ET₅₀) at pH 3.8

To determine the effects of the previous chronic exposure on subsequent pH sensitivity of fish from the various treatments, samples from each exposure group (Table 1) were exposed at pH 3.8 to determine their respective median time to death (ET₅₀). These exposures were made on days 42, 84, and 113 using 10 fish from each group (or in the case of the 84-d testing, all fish remaining, after previous sampling and treatment related mortalities, in the following treatment groups: pH 5.0, 30 μ g Al/L (B) (six fish); pH 4.5, 30 μ g Al/L (A) (eight fish); and B (seven fish). The pH 3.8 challenges were conducted at 4° C and in water of the same Ca concentration as their previous acclimation. Survival time determinations were made in tanks holding 22.7 L of constantly aerated water, divided in the center

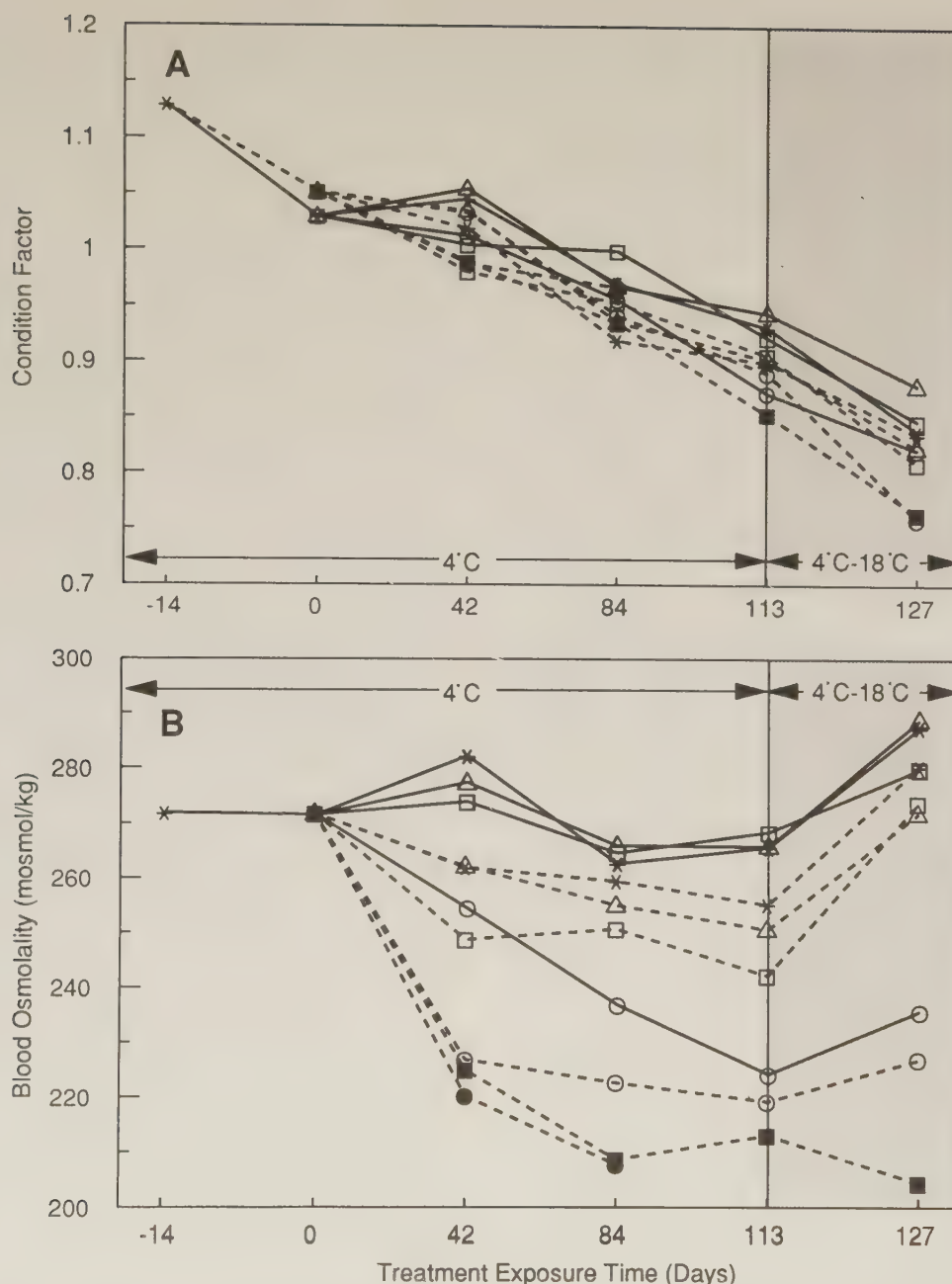


FIG. 1. Change in YOY largemouth bass (A) condition factor, (B) blood osmolality, and (C) survival probability after exposure to acid (pH 8.0 (*—*), 7.0 (*---*), 5.5 (Δ), 5.0 ($\square$), and 4.5 ($\circ$)) and Al (no added Al (open symbols) and 30 μ g monomeric Al/L (closed symbols)) in soft water (13.4 mg Ca/L) (solid lines) and very soft water (1.5 mg Ca/L) (broken lines). (Fig. 1 concluded next page)

with a stainless steel screen to allow exposure of samples of fish from two randomly assigned chronic exposure treatment groups at a time. One half of the volume of these tanks was removed and replaced daily with the appropriate fresh solution. Adjustments for pH were made twice each day to within ± 0.02 pH unit. A mean temperature of 4°C (3.5–4.6°C) was maintained by immersion of these exposure tanks in thermostatically controlled flowing chilled water baths. Mortality observations were made at hourly intervals.

Statistical Analysis

Condition factor and blood osmolality

Because replicates (tanks) were followed across time, a repeated measures design (Milliken and Johnson 1984) was

used with tanks as the experimental unit. Effects of pH, Al, Ca concentration, exposure time, and interactions between these effects were tested using a mixed-models univariate approach for repeated measures. Imbalance caused by the depletion of fish in some tanks before the end of the experiment required use of synthetic denominators in the *F*-tests (Steel and Torrie 1960). Separate ANOVA's were conducted for each date. Without the Al treatments, the design is a complete 2×3 factorial with water Ca concentration and pH; these effects were tested using this balanced design. Specific comparisons were made between Ca concentrations at the same pH (e.g. 13.4 mg Ca/L water at pH 4.5 versus 1.5 mg Ca/L water at pH 4.5) and between pH levels in the same Ca concentration water (e.g. pH 4.5 versus pH 8.0 in the 13.4 mg Ca/L water). Significance

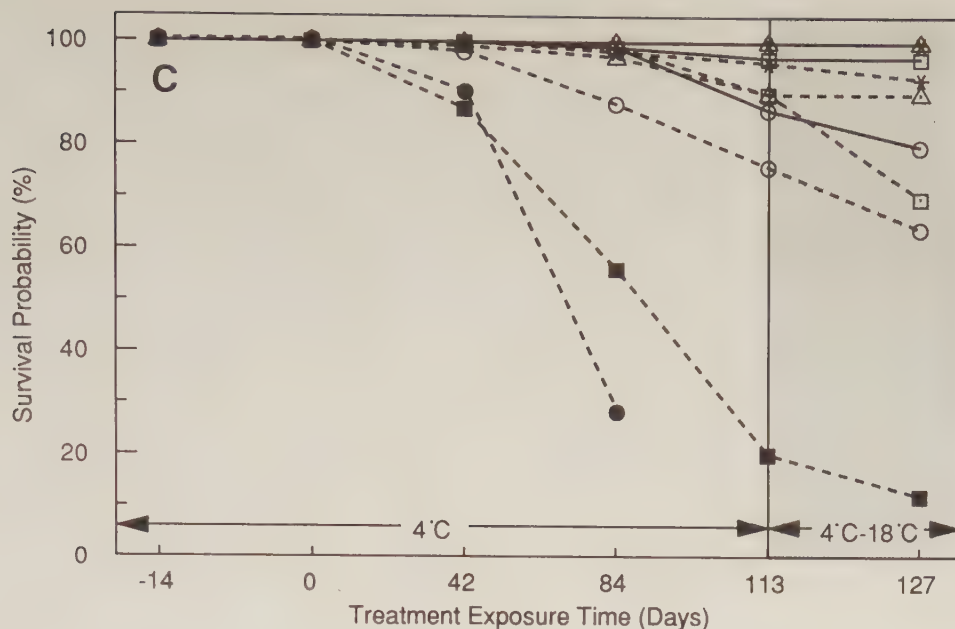


FIG. 1. (Concluded)

of the 16 contrasts was tested using a Bonferroni correction for specific multiple comparisons (Milliken and Johnson 1984). Tests for Al effects at days 42 and 84 were performed using a separate ANOVA with two contrasts comparing treatments with and without added Al at the same pH. Only the 42- and 84-d time points were used, since data for both replicates of these treatments were not available beyond the day 84.

Survival probability

During the chronic exposure experiment, in addition to mortalities, fish were removed for blood osmolality measurements and for ET_{50} determinations, requiring analysis appropriate for right-censored data (Cox and Oakes 1984). Right censorship implies that the time of death of certain fish is known only to be greater than the time when sampled. The SAS LIFETEST procedure (SAS Institute Inc. 1985) was used to compute Kaplan-Meier product limit estimates of survival probabilities across time for each separate treatment. All analyses were performed with survivals of individual fish. The significance of compared survivals in individual treatments (e.g. pH 5.0 without added Al versus pH 5.0 with added Al) was tested using the logrank test in LIFETEST. Calcium and Al effects were tested with the logrank test after pairing on the same pH's.

For the treatment at pH 4.5 with added Al, all the fish had died or had been sampled by day 85; thus the survivorship to day 84 was used to calculate the survival probability for that treatment. Relationships between survival and condition factor and between survival and osmolality were determined using the Pearson correlation coefficient (SAS Institute Inc. 1985).

Median time to death (ET_{50}) at pH 3.8

The significance of the effects due to the various chronic exposures (treatments and time) was assessed by using ANOVA procedures with log-transformed sample medians, as described above for condition factors and osmolality. Using individual fish survival times at pH 3.8 and their associated condition factors, and after checking that the assumption of parallel slopes for the different treatments was valid (Freund et al. 1986), an ANCOVA was run to estimate the common effect of condition factor on survival time.

To evaluate the effect of condition factor on survival time, an ANCOVA was performed which gives an estimate of the regression coefficient, b , relating $\log_{10}$ survival to condition factor. The estimated ratio of the survival time for condition factor, K_1 , to the survival time for condition factor, K_2 , is given by $10^{b(K_1 - K_2)}$.

Results

Condition Factor

The mean condition factor of the fish from all treatments declined over the 127 d of exposure (Fig. 1A). At the time of distribution to the exposure tanks, the mean condition factor was 1.20, similar to the 1.18 mean of fall-sampled YOY largemouth bass from Little Rock Lake (J. H. McCormick, unpublished data). Both time of exposure and water Ca content affected the final condition factor ($P < 0.01$). Aluminum concentration (at pH 5.0) had an effect only after 113 and 127 d of exposure ($P < 0.05$), i.e. condition factors became lower with longer exposure time, due to lower Ca content, and with added Al. No significant effects due to pH were detected.

Food Consumption

Food consumption by the study fish was related to exposure conditions. At 3.8°C, little feeding was observed in any treatment group. Decreased feeding was more noticeable in the 1.5 mg Ca/L exposed fish than in the 13.4 mg Ca/L exposed groups. Feeding inhibition was, however, most evident in those fish in the pH 4.5 soft and very soft water treatments and in the 4.5 and 5.0 very soft water treatments with added Al. When the temperature was increased to 18°C, food consumption was enhanced in all surviving treatment groups, except in the pH 4.5 treatments, and at pH 5.0 with added Al.

Blood Osmolality

By day 42, treatment-related changes in blood osmolality were observed ($P < 0.01$) (Fig. 1B). In general, blood osmolalities were lowered in fish from the 1.5 mg Ca/L water than

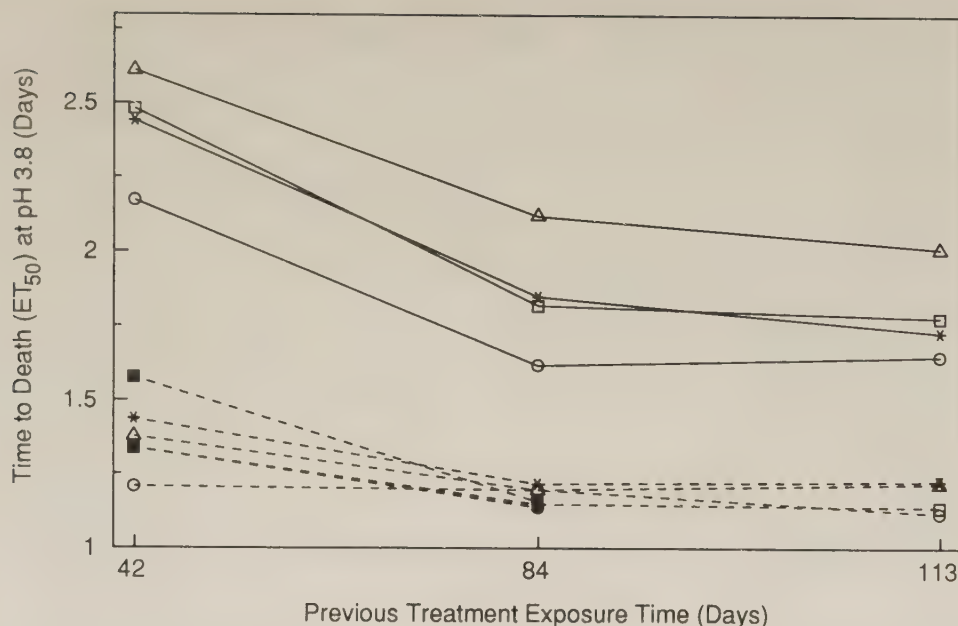


FIG. 2. Effect of prior chronic exposure for 42, 84, and 113 d on survival time (ET_{50}) of YOY largemouth bass. Exposures were conducted at 4°C and in water of the same Ca concentration as the previous chronic treatment. Refer to Fig. 1 for definition of symbols.

in those from the 13.4 mg Ca/L water. In the 1.5 mg Ca/L treatments the lowest blood osmolality values were in fish from the pH 4.5 treatment and at pH 4.5 and 5.0 with added Al (226, 220, and 225 mosmol/kg, respectively) compared with a mean of 262 mosmol/kg in fish from the pH 7.0, 1.5 mg Ca/L exposure groups. At the same time, no differences in blood osmolality were detected between fish exposed in the 13.4 mg Ca/L water at pH 5.0, 5.5, and 8.0 (274, 278, and 282 mosmol/kg, respectively). However, by 42 d the blood osmolalities of fish from the pH 4.5, 13.4 mg Ca/L water had declined to 254 mosmol/kg ($P < 0.01$) (Fig. 1B).

By day 84 of exposure the mean blood osmolalities of the largemouth bass from both very soft water with added Al treatments (pH 5.0 and 4.5) had further declined to the near lethal threshold value (McCormick et al. 1989b) of 208 and 209, respectively. The value for the pH 4.5 group without added Al was also low, 223 mosmol/kg (Fig. 1B), but not yet life threatening. Among the 13.4 mg Ca/L exposed fish, only the pH 4.5 group had fallen significantly, to 237 mosmol/kg ($P < 0.01$). Little further change in blood osmolality was observed between the 84- and 113-d readings in the fish from the 13.4 mg Ca/L water, except in the pH 4.5 groups, where the osmolality continued to fall until it reached 224 mosmol/kg by day 113. Between 113 and 127 d of exposure, accompanying the spring-like temperature rise to 18.0°C, all but the most debilitated remaining group (pH 5.0 with added Al) responded with a rise in osmolality (Fig. 1B).

Survival Probability

Survival probability to day 127 was affected ($P < 0.01$) by length of exposure and the Ca, pH, and Al concentration of the exposure water (Fig. 1C). The most severe effects were observed in those groups receiving added Al, with significantly lower probabilities of survival compared with fish from equivalent Ca and pH treatments without added Al. For example, at pH 5.0 without added Al, there was a 99% probability of survival to day 84, but only a 56% probability when Al was added.

The probabilities of survival to the day 84 at pH 4.5 and 5.0 in the 13.4 mg Ca/L exposures were 87 and 97%, respectively. Toward the end of the study, one replicate of the pH 4.5, 13.4 mg Ca/L group had some obviously stressed fish and mortalities were observed.

The stress syndrome preceding death began with a general lethargy, and some individuals in the more stressful treatments progressed from lethargy to cessation of feeding to lying on their sides on the bottom. These individuals were generally emaciated and death would be expected several days later. When one of these moribund fish (treatment pH 5.0 with added Al) was sampled on day 42 of exposure, its blood osmolality was 166 mosmol/kg.

Probability of survival was correlated with blood osmolality ($r = 0.87$, $P < 0.01$) but not with condition factor ($r = 0.36$, $P > 0.30$).

Median Time to Death (ET_{50}) at pH 3.8

When fish from the chronic exposures were transferred to pH 3.8, the ET_{50} values were shorter after preexposure to test conditions of longer duration, lower pH, and reduced Ca concentrations ($P < 0.01$) (Fig. 2). Effects on ET_{50} values due to previous chronic exposure to pH 4.5, 5.0, and 5.5 were most readily identified in fish from the 13.4 mg Ca/L water, where differences were found only between responses of fish previously exposed at pH 5.5 and those held at 4.5 on days 84 and 113 ($P < 0.01$) (Fig. 2). The pH 4.5, 5.0, and 5.5 treatment groups from the 13.4 mg Ca/L water had progressively shorter ET_{50} values with decreasing pH ($P < 0.01$), but the pH 8.0 group was not significantly different from the pH 5.0 group, perhaps because of a greater transfer shock from pH 7.8 to 3.8. Survival times in the very soft water groups were shorter than in those in the soft water groups ($P < 0.01$), but no between-treatment effects were found in the very soft water groups. ET_{50} values of fish were related to the condition factor at the time of exposure, i.e. fish with a mean condition factor of 1.0 could

be expected to live 68% longer than those with a condition factor of 0.5.

During transfer of fish from the chronic treatments to the pH 3.8 challenge tanks, a convulsive tetanus-like behavior was observed in several fish taken from each of the chronic treatment groups with added Al. This tetany was particularly noticeable on day 42. The fish quivered and sank to the bottom of the transfer beakers, and this behavior continued for a time after transfer to the ET₅₀ tanks. However, most fish recovered from this shock before eventually dying of the pH 3.8 exposure.

Discussion

If loss of energy stores alone affected survival, the mortality rates of the fish in all treatments would have been expected to follow more faithfully the path of the decline of their respective condition factors; this was not the case. Therefore, it is more likely that the mortalities observed in this study were due to loss of ionoregulatory control than to exhaustion of energy reserves. The decline in condition factor among the fish from all the treatments followed a similar path (Fig. 1A), while the probability of survival was closely allied to the decline in blood osmolality of the fish in the respective treatments (Fig. 1B and 1C).

These observations do not necessarily refute the view that depletion of energy reserves is the cause of overwintering mortalities in many fish populations (Reimers 1963; Newsome and LeDuc 1975; Toney and Coble 1979; Shuter et al. 1980, 1989; Henderson et al. 1988). They do, however, suggest that in acidified very soft waters, where Al is elevated, overwintering mortalities due to depletion of essential ions or their unavailability (R. L. Leino and J. H. McCormick, School of Medicine, University of Minnesota, Duluth, MN, unpubl. data) may occur before energy reserves become critically low (Toney and Coble 1980). Mineral losses in fishes exposed to low pH (Fraser and Harvey 1982) or due to fasting (Van Someren 1937; Ichikawa 1953; Yamada 1956) have previously been reported. Calcium loss from fish scales during periods of extended fasting has been specifically reported by these authors. Some additional factors contributing to the greater rate of vital ion loss in the affected treatment groups may have been the paucity of ions available in the 1.5 mg Ca/L water (Phillips et al. 1961) or near cessation of feeding by the fish at 3.8°C (Phillips et al. 1956; Sadler and Lynam 1986; Smith et al. 1989; Duston et al. 1991). Recent work by R. L. Leino and J. H. McCormick (School of Medicine, University of Minnesota, Duluth, MN, unpubl. data), using fish from this same experiment, also showed that Ca reserves present in the scales of fish from environments with elevated Al may be less accessible in times of homeostatic demand (Urist 1962; Mugiya and Watabe 1977). In addition, ion losses are accelerated by increased membrane permeability at colder temperatures (Houston et al. 1968; Davis and Simco 1976) and low Ca levels (Wood and McDonald 1982; Neville 1985).

Fish suffering from Ca deficiency may thus experience disruption of several essential biological processes (Carafoli and Penniston 1985), including acid-base balance, regulation of nerve conduction, control of membrane permeability (Potts and McWilliams 1989), and enzyme inactivation due to inadequate Ca as a cofactor (Neuhold and Sigler 1954). Because Al interferes with many Ca-dependent functions (Dunstan et al. 1985; Playle et al. 1989; Reid et al. 1991) and because Al is more soluble and toxic below pH 6 (May et al. 1979; LaZerte 1984; Neville 1985), environmental acidification may further aggra-

vate Ca-related dysfunctions by its influence on Al availability (Kruck and McLachlan 1988).

The tetany syndrome exhibited by fish from the added Al treatments, when transferred from chronic to acute exposure tanks, suggests Ca insufficiency (Neuhold and Sigler 1954; Turner 1960; Pang et al. 1971). That this effect was observed only in those treatments with added Al is evidence of the antagonism between Al and Ca for vital physiological processes (Tam and Williams 1986; Booth et al. 1988). The chronic exposure effects on osmoregulation at low pH again affirm that pH is a prime factor in ion losses and in acid-related mortalities of freshwater fishes (Wood 1989), particularly when the lower pH results in mobilization of Al (Christoffersen and Christoffersen 1985; Battram 1988) and shifts its speciation to more toxic forms (Neville 1985).

The importance of temperature in these responses is accentuated when it is noted that no pH-related mortalities or loss of blood osmolality were observed when YOY largemouth bass were exposed to pH 4.5 water for 30 d at 20°C (McCormick et al. 1989b). Cold temperature (Meyer et al. 1956; Eddy 1981) which greatly reduced food intake (Bilton and Robins 1971; Jürss et al. 1983; Virtanen and Soivio 1985; Bigler 1989) and ion-poor water (Colby 1973) combined to lower ion influx well below that required to compensate for the accelerated efflux of essential ions at low pH, especially when Al is elevated. Further support for the importance of temperature in ionoregulation was the increase in blood osmolality in all but the most severely debilitated treatment groups, when the temperature was raised from 3.8 to 18°C. Mortalities of exposed fish did, however, continue through this period (Fig. 1C). Some individuals had apparently exceeded their tolerance limit for recovery before the temperature was increased.

Kwain et al. (1984) found that smallmouth bass survival time at low pH became shorter after enforced fasting and that their 96-h LC₅₀ pH became progressively higher as the fasting time was extended. If a similar response relationship between ET₅₀ and LC₅₀ occurs in largemouth bass, their sensitivity to low pH would be expected to increase as winter fasting proceeds (Cunningham and Shuter 1986). Consequently, a pH tolerated by fish at other times of the year may become acutely lethal after they have endured the winter. If such is the case, it is important to note that it is at the end of winter that acid pulses are most consistently encountered (Gjessing et al. 1976; Jeffries et al. 1979; Siegel 1981).

In the field study involving Little Rock Lake largemouth bass, seasonal conditions were equal between the acid-treated and the reference basins. The YOY largemouth bass of Little Rock Lake appeared to have cleared all of the necessary hurdles for establishing an age 1 year class when they entered their first winter at pH 5.1, i.e. spawning had occurred, the embryos had hatched, and juveniles of nearly equal sizes and abundance were present in the fall in both the acid-treated and reference basins (Eaton et al., (U.S. EPA), Environmental Research Laboratory-Duluth, Duluth, MN, unpubl. data). However, the 1987 year class failed in the acid-treated basin during overwintering, while they were well represented as age 1 fish in the reference basin the following spring. Therefore, acidification of the soft cold water of that lake is thought to have had an overwintering effect beyond that anticipated from the length of the winter alone. The findings from this laboratory study indicate that high overwintering mortality can be expected among age 0–1 largemouth bass in very soft water when the pH falls to 5.0 or less, if, at the same time, monomeric Al is also elevated to $\geq 30 \mu\text{g/L}$. Yet, those larger individuals who survive their first

winter may persist from year to year due to their larger relative reserves of essential resources and lower metabolic demands.

Possibly the most important finding of this study was that overwintering of age 0–1 largemouth bass can be the critical period for year class recruitment even though the embryo-larval stage may be more sensitive in a toxicological sense. Contributing to such overwintering vulnerability of YOY largemouth bass in northern waters are reduced growth rates, shortened growing seasons, and lengthened overwintering time. Also, environmental acidification accelerates ion losses and mobilizes environmental Al which aggravates these effects in poorly buffered low-Ca waters. As a result, when environmental acidification occurs near the northern limits of distribution of largemouth bass, recruitment of age 1 fish will become progressively less dependable until the species is no longer able to sustain itself, thus moving its northern limit of distribution southward.

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Effects of Nutrient Enrichment and Flood Frequency on Periphyton Biomass in Northern Ozark Streams¹

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Lohman, K., J. R. Jones, and B. D. Perkins. 1992. Effects of nutrient enrichment and flood frequency on periphyton biomass in northern Ozark streams. *Can. J. Fish. Aquat. Sci.* 49: 1198–1205.

Ambient nutrient concentrations (TN and TP) and periphyton biomass (Chl *a*) were measured every 2 wk during March–November in 1985 and 1986 at 22 sites on 12 streams in the northern Ozarks, Missouri. Benthic Chl *a* was positively correlated in both years with log TN ($R^2 = 0.58, 0.60$) and with log TP ($R^2 = 0.47, 0.60$). When sites were grouped by the degree of enrichment and plotted over time, benthic Chl *a* decreased at all sites after flood events, but rebounded more rapidly at highly enriched sites. Differences in recovery following flooding were most obvious in fall 1986, when both high and moderately enriched sites exhibited similar biomass accrual patterns, reaching mean benthic Chl *a* of 397.4 and 321.1 $\text{mg}\cdot\text{m}^{-2}$, respectively, within 42 d after a catastrophic flood. In contrast, average benthic Chl *a* at nutrient-poor sites reached a maximum level of 76.8 $\text{mg}\cdot\text{m}^{-2}$ within 28 d after flooding, suggesting that maximum standing crops are influenced by both nutrient supply and the length of the flood-free period.

Les concentrations ambiantes en matières nutritives (NT et PT) et la biomasse de périphyton (Chl *a*) ont été mesurées toutes les deux semaines en 1985 et 1986, de mars à novembre, en 22 endroits situés dans 12 cours d'eau du nord des Ozark, au Missouri. On a établi une corrélation positive, au cours de ces deux années, entre la Chl *a* benthique et le log NT ($R^2 = 0.58, 0.60$) et le log PT ($R^2 = 0.47, 0.60$). Après avoir regroupé les données en fonction de l'enrichissement en matières nutritives et tracé les courbes correspondant aux résultats en fonction du temps, on a noté une diminution de la Chl *a* benthique dans tous les sites à la suite d'inondations, mais ce taux a augmenté plus rapidement aux endroits très riches en matières nutritives. Les différences de récupération à la suite d'inondations ont été plus évidentes à l'automne de 1986, où les sites fortement et modérément enrichis ont présenté des profils similaires d'accroissement de la biomasse, pour atteindre respectivement la concentration moyenne de Chl *a* benthique, soit 397,4 et 321,1 $\text{mg}\cdot\text{m}^{-2}$, dans les 42 jours suivant une inondation catastrophique. Par contraste, la Chl *a* benthique dans des sites pauvres en matières nutritives a atteint un niveau maximal de 76,8 $\text{mg}\cdot\text{m}^{-2}$ dans les 28 jours suivant une inondation, ce qui laisse à penser que les populations exploitables sont tributaires de la disponibilité en matières nutritives ainsi que de la durée des périodes exemptes d'inondation.

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Nutrient enrichment experiments have demonstrated that nitrogen or phosphorus additions can stimulate periphyton growth in streams (Stockner and Shortreed 1978; Peterson et al. 1983; Grimm and Fisher 1986; Lohman et al. 1991). In regional studies, however, attempts to explain differences in periphyton biomass among streams by differences in nutrient concentrations have not shown clear relationships. Correlations between periphyton biomass and ambient nutrient concentrations have been noted among streams within regions in Japan (Aizaki and Sakamoto 1988) and New Zealand (Biggs and Close 1989), but no such relationship was found in two studies in the United States (Jones et al. 1984; Welch et al. 1988). Thus, whereas models relating phytoplankton biomass and nutrients have been highly successful lake management tools (e.g. Dillon and Rigler 1974; Jones and Bachmann 1976; Canfield 1983), empirical correlation may not be a reliable method for establishing nutrient guidelines in streams (Welch et al. 1988, 1989).

The importance of disturbance in stream ecosystems may explain why strong nutrient–biomass relationships have not been found in stream studies. Disturbance, most often by flooding, has been described as the dominant organizing factor in streams (Resh et al. 1988), and the dramatic effect of severe floods on periphyton biomass has been well documented (Douglas 1958; Tominaga and Ichimura 1966; Sumner and Fisher 1979; Rounick and Gregory 1981; Freeman 1986; Power and Stewart 1986; Fisher and Grimm 1988; Biggs and Close 1989; Grimm and Fisher 1989). In regions where streams are subject to large and frequent flood events, the effects of nutrient enrichment on periphyton growth may be obscured by resetting floods that prevent biomass accrual. If so, the length of flood-free periods may be the single most important factor in determining levels of periphyton biomass (Tett et al. 1978; Fisher and Grimm 1988; Biggs and Close 1989; Grimm and Fisher 1989).

Grimm and Fisher (1986) have hypothesized that nutrients regulate periphyton accrual in streams but that maximum standing crops are primarily a function of flood frequency. They predicted that, in the absence of other limiting factors, rates of biomass accrual should be greater in nutrient-rich than in nutrient-poor streams but that given sufficient time without a resetting flood, periphyton biomass in all streams should be

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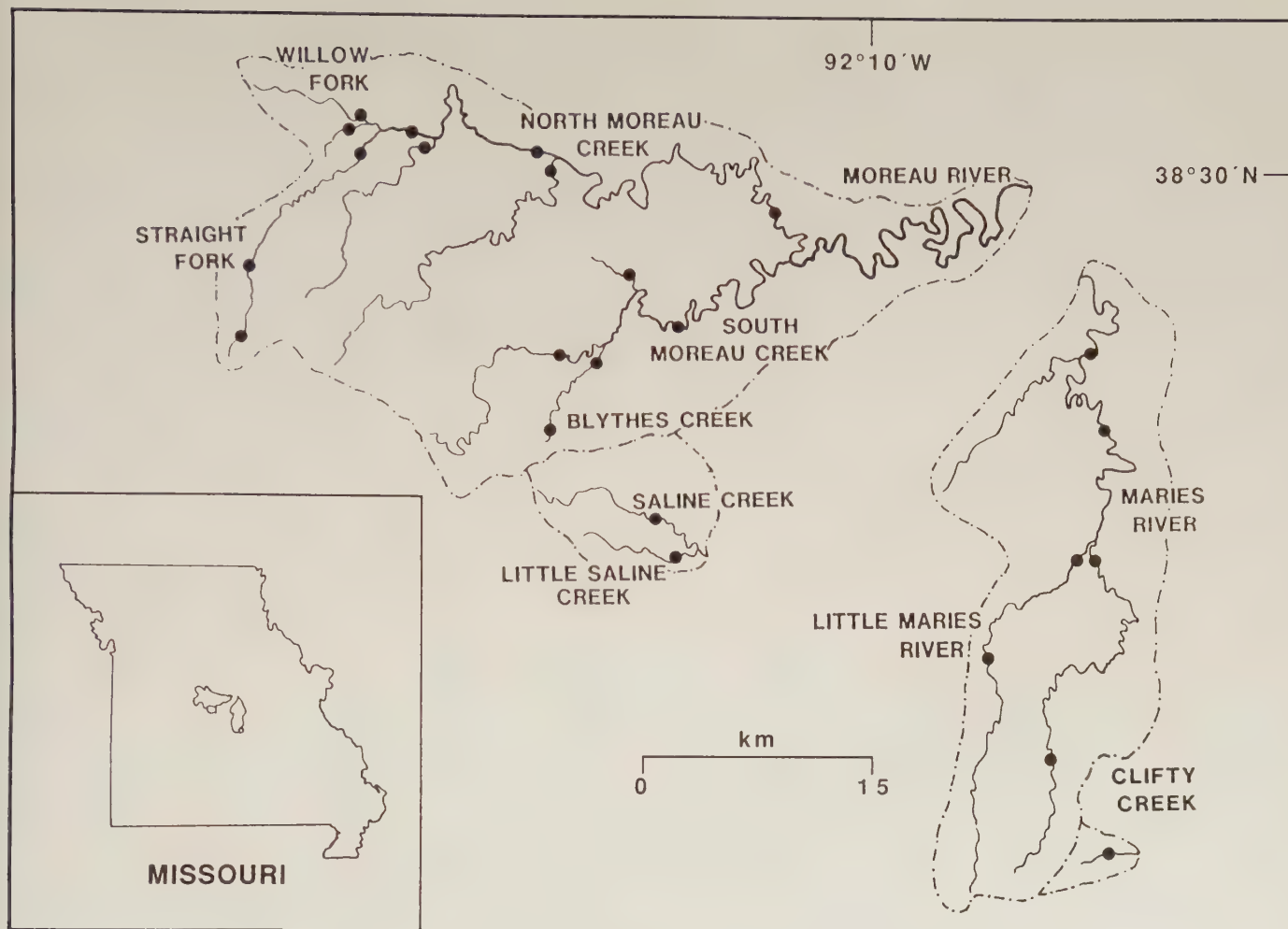


FIG. 1. Twenty-two stream sites in the northern Ozark Plateau sampled during 1985-86.

similar. This hypothesis suggests that whether differences in biomass among streams can be explained by differences in nutrients may well depend on both the interval between floods and the ambient nutrient concentrations. Detecting a nutrient effect on periphyton growth would seem most likely in a region where streams have a broad range of nutrient regimes and where flood-free periods are sufficient for substantial biomass accrual, but not so long that factors other than nutrients limit biomass.

In this study, we investigated the relation of nutrients to periphyton biomass in streams of the northern Ozarks. Our objectives were to determine whether differences in periphyton biomass among streams could be explained by differences in ambient nutrient concentrations and to relate temporal changes in periphyton biomass to nutrients and flood frequency. A catastrophic flood, followed by a prolonged flood-free period, also allowed us to conduct a fortuitous natural experiment comparing rates of periphyton accrual among streams with varying nutrient concentrations and to test the predictions of Grimm and Fisher's (1986) model of biomass accrual.

Description of Study Sites

Nutrient and periphyton data were collected over a 2-yr period at 22 sites on 12 streams in the northern Ozarks, Missouri (Fig. 1). Nineteen sites were located within the drainage basins of the Maries and Moreau rivers, two of the larger streams draining the region. The Maries River is a fourth-order

tributary of the Osage River and the Moreau River is a fifth-order tributary of the Missouri River. Three other sites were located within two watersheds (Saline and Clifty creeks) adjacent to the Maries and Moreau river drainage basins.

Drainages of the Maries and Moreau rivers reflect the transitional nature of the northern Ozark region. The area drained by the Maries River is characterized by deeply dissected topography, similar to the Ozark Plateau, whereas the Moreau River watershed more closely resembles the till plains of northern and western Missouri. Bedrock geology in both watersheds is predominantly Jefferson City limestone and dolomite of lower Ordovician age (Collier 1953; Missouri Department of Natural Resources 1984). Forest covers greater than 50% of the Maries River watershed; row crop and pasture predominate in the Moreau River watershed. Urban area makes up less than 5% of both watersheds. Presettlement vegetational cover was forest in the Maries River watershed and a mosaic of forest and prairie in the Moreau River watershed (Schroeder 1981).

Methods

Sample Collection, Water Chemistry, and Chlorophyll *a* Analysis

Sites were visited over three consecutive days every 2 wk during March-November in 1985 and 1986. A composite water sample, from middepth at three intervals across the stream, was collected at each site. Thirty-eight samples were collected at

TABLE 1. Mean annual TP, TN, and benthic Chl *a* at 22 sites on 12 streams in the northern Ozarks. Sites were classified as high, moderate, or unenriched based on TP concentrations, with the exception of Maries River, site 1, which was classified as moderately enriched based on known point-source additions not reflected in the ambient nutrient concentrations.

	TP ($\mu\text{g}\cdot\text{L}^{-1}$)		TN ($\mu\text{g}\cdot\text{L}^{-1}$)		Benthic Chl <i>a</i> ($\text{mg}\cdot\text{m}^{-2}$)	
	1985	1986	1985	1986	1985	1986
High enrichment						
Blythes Creek						
Site 1	3264	2923	9188	7682	216.7	182.2
Site 2	288	411	947	1024	100.6	146.9
Straight Fork						
Site 1	2447	1825	4509	3758	67.6	113.9
Site 2	554	395	1484	999	123.9	107.7
Willow Fork	1073	713	2080	2116	56.4	83.4
North Moreau Creek						
Site 2	212	240	872	974	46.8	70.0
Moderate enrichment						
South Moreau Creek						
Site 1	52	47	450	467	29.2	61.0
Site 2	79	93	515	436	59.9	85.9
Straight Fork						
Site 3	104	62	574	521	76.8	81.6
Smiths Creek	81	44	643	414	35.9	54.1
Burris Fork	70	70	428	614	28.4	77.3
North Moreau Creek						
Site 1	101	82	603	498	30.6	41.5
Site 3	98	125	621	773	37.7	30.1
Little Saline Creek	30	28	516	550	50.8	86.6
Maries River						
Site 1	17	14	276	186	37.0	78.6
Site 4	32	34	319	306	39.9	71.6
Low enrichment						
Maries River						
Site 2	16	19	304	284	39.9	44.4
Site 3	15	19	329	269	59.8	50.9
Little Maries River						
Site 1	14	13	229	210	54.9	45.0
Site 2	15	15	283	259	33.2	39.9
Saline Creek	19	19	199	166	31.3	39.4
Clifty Creek	6	6	148	159	10.3	20.1

each of the 22 sites over the 2-yr period. For convenience, values averaged across the March–November period are referred to as mean annual values.

Nutrient concentrations were measured as total phosphorus (TP) and total nitrogen (TN). TP was determined using the ascorbic acid method after persulfate oxidation (Prepas and Rigler 1982). TN was analyzed by cadmium reduction after persulfate oxidation (D'Elia et al. 1977). Periphyton samples were collected on each sampling date for analysis of chlorophyll *a* (Chl *a*). Periphyton within a 5.3-cm² area was scraped from five rocks collected along a transect across a riffle at each site. Each scraping was washed into a scintillation vial with distilled water and later rinsed onto a Gelman type A/E glass fiber filter. Chl *a* was extracted by placing filters in a 50:50 mixture of dimethyl sulfoxide (DMSO) and 90% acetone in the dark for 24 h (Shoaf and Lium 1976). After extraction, Chl *a* was determined fluorometrically and corrected for phaeopigments by acidification (Knowlton 1984). Samples analyzed before July 1985 were not acidified; however, a good correlation was found between Chl *a*, corrected for phaeopigments, and unacidified samples analyzed between July 1985 and November 1986 ($\text{Chl } a_{\text{acidified}} = 0.41 + 0.86 \text{ Chl } a_{\text{unacidified}}$, $R^2 = 0.98$,

$p \leq 0.0001$, $n = 2820$). This equation was used to estimate Chl *a*, corrected for phaeopigments, from unacidified samples analyzed before July 1985.

Regression Analysis

Mean annual density of benthic Chl *a* and mean annual concentrations of TN and TP were calculated for each site. Chl *a* values $< 2.0 \text{ mg}\cdot\text{m}^{-2}$ were considered the result of recent flooding and were not included in calculating means. To normalize nutrient concentrations, log-transformed values of mean TP and TN were used in linear regressions of each nutrient against Chl *a*. Separate models were constructed for each year and covariance analysis used to test for differences between years.

Temporal Patterns in Benthic Chl *a*

Based on mean annual TP concentrations (Table 1), sites were classified as high enrichment (six sites affected by secondarily treated sewage effluent), moderate enrichment (10 sites, most receiving some enrichment from agricultural land use activities), and low enrichment (six sites draining primarily

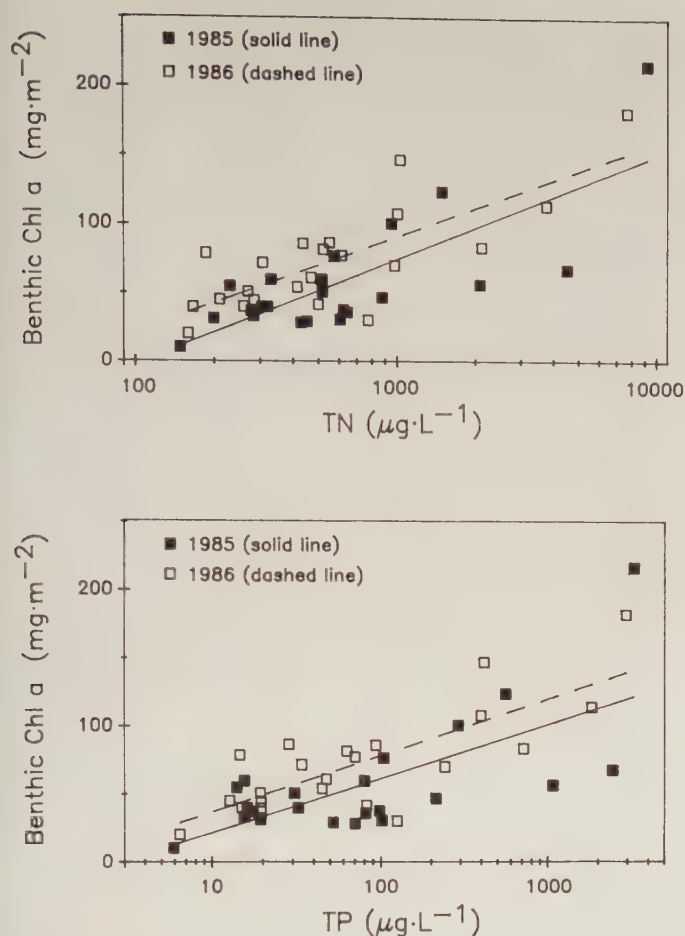


FIG. 2. Regressions of log TN and log TP against benthic Chl *a* using annual means (March–November) for 1985 and 1986 from 22 stream sites in the northern Ozark Plateau.

forested watersheds). One site (Maries River, site 1), which would have been characterized as low enrichment based solely on TP concentration, was placed in the moderately enriched category. Chloride and sulfate concentrations 1.5–3 times higher than at downstream sites on the Maries River indicated that chemical conditions at this site were influenced by a wastewater discharge located 20 km upstream and that TN and TP supply were likely higher than that suggested by ambient concentrations. Mean annual TP was $212\text{--}3264\ \mu\text{g}\cdot\text{L}^{-1}$ in enriched sites, $14\text{--}125\ \mu\text{g}\cdot\text{L}^{-1}$ in moderately enriched sites, and $6\text{--}19\ \mu\text{g}\cdot\text{L}^{-1}$ in sites of low enrichment. Mean benthic Chl *a* on each sampling date was calculated for each level of enrichment and plotted over time. Because bimonthly sampling was conducted over 3-d periods, not all sampling reflected similar flood histories. To illustrate the effects of nutrient enrichment on periphyton biomass in the context of flood frequency, benthic Chl *a* over time was compared from sites on three streams (Blythes, Little Saline, and Saline creeks) considered representative of the high-, moderate-, and low-nutrient classifications. These streams were sampled on the same dates. Their watersheds are adjacent, receive similar rainfall, and are subject to similar flood frequencies. Weekly rainfall, based on data from a recording station located near the source of all three streams (NOAA 1985, 1986), was used as a measure of flood frequency.

Postflood Recovery of Periphyton

Decimation of periphyton standing crops following a catastrophic flood allowed us to compare postflood recovery of

periphyton among stream sites of high, moderate, and low nutrient enrichment. Rainfall of 30–40 cm within a 2-wk period in September–October 1986 occurred throughout the northern Ozarks (NOAA 1986), and streams at all study sites were bank-full or out of their banks during the first week in October. Benthic Chl *a* at 20 of 22 sites, measured within 2–3 d after flood waters receded, was $<0.1\ \text{mg}\cdot\text{m}^{-2}$. We continued to measure Chl *a* at 2-wk intervals for 6 wk following the flood, during which time no substantial increase in discharge occurred. Growth curves for groups of high-, moderate-, and low-nutrient sites were fitted by polynomial regression in which the intercept term was omitted.

Results

Regression Analysis

Benthic Chl *a* was positively correlated with log-transformed concentrations of TN in both 1985 and 1986 (Fig. 2):

$$(1) \text{ Chl } a = 76.9 \log \text{ TN} - 155.8 \\ R^2 = 0.58, p \leq 0.001, n = 22 \text{ (1985)}$$

$$(2) \text{ Chl } a = 69.3 \log \text{ TN} - 116.7 \\ R^2 = 0.60, p \leq 0.001, n = 22 \text{ (1986).}$$

Covariance analysis indicated that the slopes were not significantly different between years ($F = 0.15, p = 0.70$) but that intercepts were significantly different ($F = 4.93, p = 0.03$). Similar relationships were present between Chl *a* and log TP (Fig. 2):

$$(3) \text{ Chl } a = 39.9 \log \text{ TP} - 18.1 \\ R^2 = 0.47, p \leq 0.001, n = 22 \text{ (1985)}$$

$$(4) \text{ Chl } a = 41.1 \log \text{ TP} - 4.1 \\ R^2 = 0.60, p \leq 0.001, n = 22 \text{ (1986).}$$

Slopes of these equations did not differ significantly ($F = 0.22, p = 0.90$) but intercepts were different between years ($F = 3.73, p = 0.06$). Higher intercepts in both Chl *a*–log TN and Chl *a*–log TP regressions reflect higher overall levels of benthic Chl *a* in 1986 than in 1985.

Temporal Patterns in Benthic Chl *a*

Benthic Chl *a* was consistently greater at sites classified as highly enriched than at sites of low and moderate enrichment throughout most of 1985–86 (Fig. 3). Greatest benthic Chl *a* was seen during spring and fall in both years at all three types of sites. During spring and fall, average benthic Chl *a* commonly exceeded $150\ \text{mg}\cdot\text{m}^{-2}$ at highly enriched sites. Maximum Chl *a* measured at individual sites over 1985–86 was $41.5\text{--}678.1\ \text{mg}\cdot\text{m}^{-2}$ and the frequency of benthic Chl *a* $>150\ \text{mg}\cdot\text{m}^{-2}$ ranged from 0 to 42.1% (Table 2).

Temporal patterns in benthic Chl *a* among the three streams with similar flood histories were also characterized by peaks in spring and fall (Fig. 4). High rainfall caused intense flooding in November 1985 and late September–October 1986, resulting in benthic Chl *a* $<1.0\ \text{mg}\cdot\text{m}^{-2}$ at all three streams. Lesser rainfall events in June 1985 and April, May, and July 1986 also coincided with large reductions in benthic Chl *a*. Postflood recovery of benthic Chl *a* was always most rapid in the highly enriched stream (Blythes Creek) and slowest in nutrient-poor Saline Creek. Over the 2-yr sampling period, benthic Chl *a* $>150\ \text{mg}\cdot\text{m}^{-2}$ occurred on nine of 38 sampling dates at Blythes Creek and on four dates at moderately enriched Little

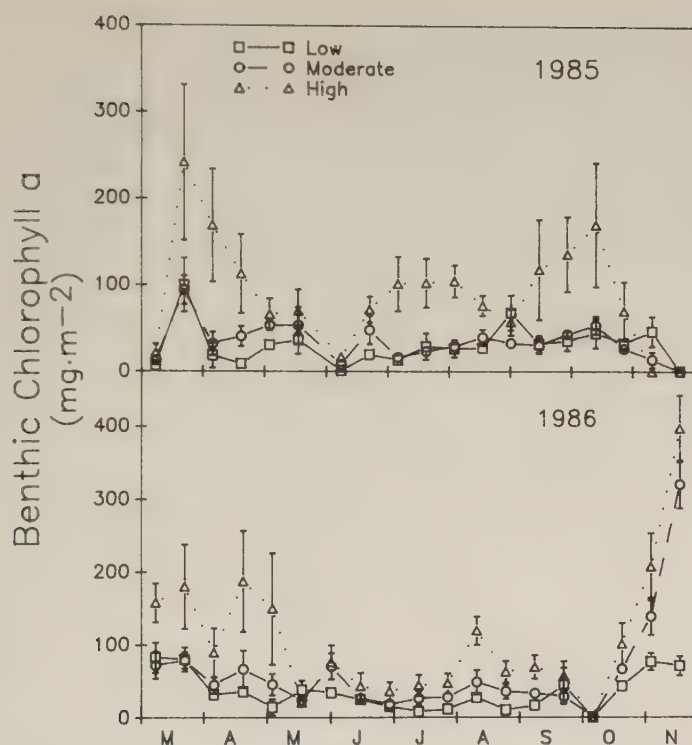


FIG. 3. Temporal changes in benthic Chl *a* in stream sites of high, moderate, and low nutrient enrichment in the northern Ozark Plateau. Values are means $\pm$ SE; $n = 6$ for high- and low-enrichment sites and $n = 10$ for moderate-enrichment sites.

Saline Creek. The maximum level of benthic Chl *a* recorded at Saline Creek was $109.0 \text{ mg} \cdot \text{m}^{-2}$.

Periphyton Accrual after a Catastrophic Flood

Patterns of periphyton accrual after a catastrophic flood in fall 1986 were similar at high- and moderately enriched sites (Fig. 5). Recovery of benthic Chl *a* at these sites was well described by second-order polynomial regressions:

High enrichment:

$$(5) \text{ Chl } a = 4.88t + 0.11t^2 \\ R^2 = 0.89, F = 89.9, p \leq 0.0001$$

Moderate enrichment:

$$(6) \text{ Chl } a = 1.75t + 0.14t^2 \\ R^2 = 0.89, F = 144.5, p \leq 0.0001$$

where t = days after flooding. Regressions for high- and moderately enriched sites differed from that for low-nutrient sites which included a negative quadratic term:

Low enrichment:

$$(7) \text{ Chl } a = 4.26t - 0.06t^2 \\ R^2 = 0.87, F = 71.8, p \geq 0.0001.$$

Covariance analysis indicated that all three curves were significantly different from one another (high versus moderate, $F = 4.98, p = 0.03$; high versus low, $F = 18.08, p < 0.0001$; moderate versus low, $F = 16.27, p = 0.0002$).

Discussion

Periphyton biomass was highly correlated with ambient concentrations of both TN and TP in northern Ozark streams. Our results contrast with Welch et al. (1988), where no relationship

TABLE 2. Percent of benthic Chl *a* samples exceeding 75 and $150 \text{ mg} \cdot \text{m}^{-2}$ and maximum benthic Chl *a* observed at 22 sites on 12 streams in the northern Ozarks during March–November 1985 and 1986 ($n = 38$ for each site).

	Percent of samples		Maximum benthic Chl <i>a</i> (mg·m ⁻²)
	>75 mg·m ⁻²	>150 mg·m ⁻²	
High enrichment			
Blythes Creek			
Site 1	65.8	42.1	678.1
Site 2	57.9	23.7	525.9
Straight Fork			
Site 1	42.1	15.8	393.8
Site 2	60.5	8.4	437.2
Willow Fork	28.9	5.3	341.4
North Moreau Creek			
Site 2	18.4	7.9	321.4
Moderate enrichment			
South Moreau Creek			
Site 1	13.2	2.6	367.2
Site 2	28.9	7.9	285.0
Straight Fork			
Site 3	36.8	7.9	440.6
Smiths Creek	10.5	2.6	220.0
Burris Fork	13.2	7.9	332.2
North Moreau Creek			
Site 1	10.5	2.6	235.8
Site 3	10.5	0.0	149.0
Little Saline Creek	31.2	10.5	335.7
Maries River			
Site 1	23.7	5.3	348.1
Site 4	13.2	5.3	498.7
Low enrichment			
Maries River			
Site 2	15.8	0.0	142.5
Site 3	23.7	2.6	272.1
Little Maries River			
Site 1	15.8	0.0	144.0
Site 2	15.8	0.0	122.5
Saline Creek	7.9	0.0	109.0
Clifty Creek	0.0	0.0	41.5

was found between mean summer Chl *a* and ambient soluble reactive phosphorus in six western Washington streams, and with Jones et al. (1984), where neither N nor P could account for variation in benthic Chl *a* among streams in the Ozark Plateau. Our findings are consistent, however, with Aizaki and Sakamoto (1988) who found that periphyton biomass was highly correlated with TN and TP in 12 Japanese streams.

Biggs and Close (1989) found a strong correlation ($r = 0.74$) between geometric annual means of Chl *a* and TP in nine New Zealand streams. For comparison with our data, we log-transformed their TP data and regressed them against Chl *a*:

$$(8) \text{ Chl } a = 47.6 \log \text{ TP} - 31.9 \\ R^2 = 0.57, p = 0.02, n = 9.$$

An analysis of covariance, including data from Biggs and Close (1989) with both years of our data in one model, indicated that neither the slope nor the intercept of the regression equation for New Zealand streams was significantly different from the equations derived for Chl *a* – log TP in northern Ozark streams. The similarity of these regressions, as well as the highly significant correlations, suggests that empirical models may prove useful in assessing stream periphyton–nutrient dynamics under some circumstances.

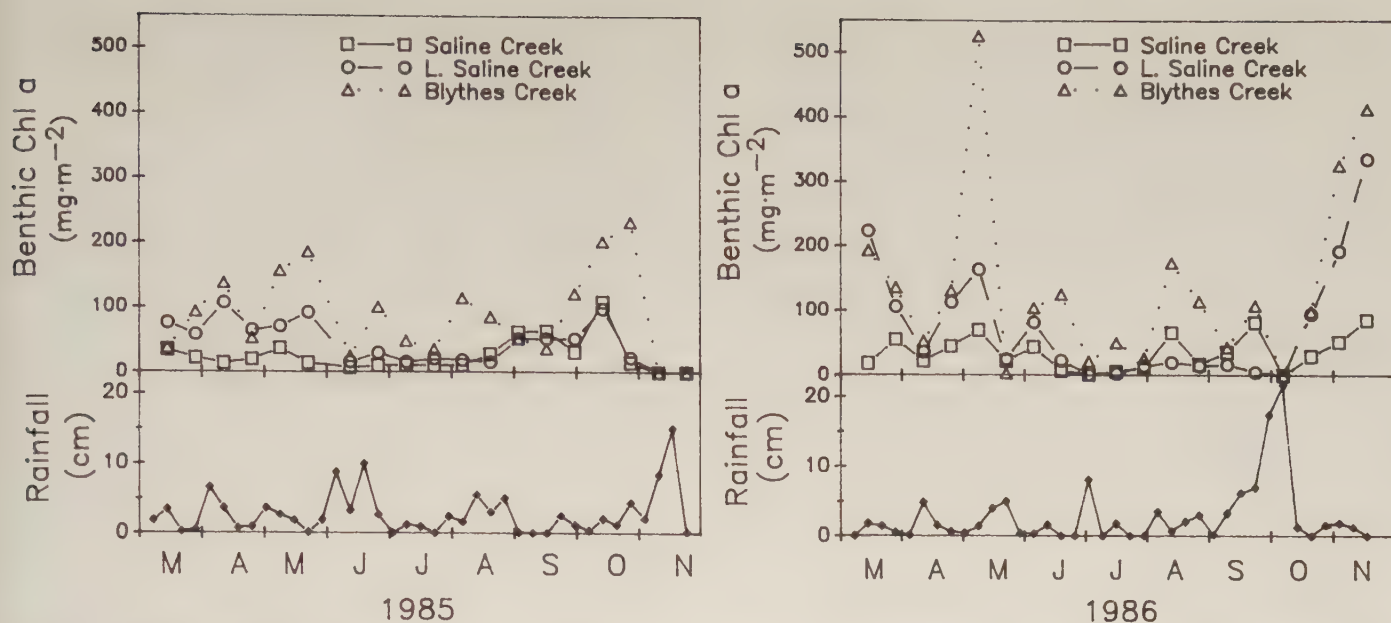


FIG. 4. Temporal changes in benthic Chl *a* in three streams representative of high (Blythes Creek), moderate (Little Saline Creek), and low nutrient enrichment (Saline Creek). Rainfall is the amount occurring during the preceding 7 d.

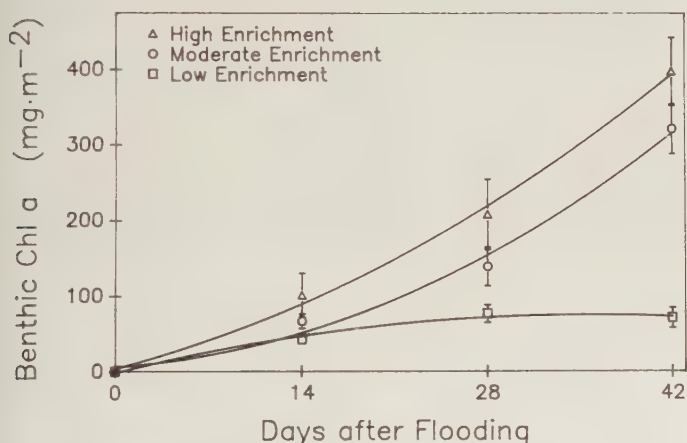


FIG. 5. Growth curves fitted for stream sites classified as high-, moderate-, and low-nutrient sites following a catastrophic flood in fall 1986. Points are means $\pm$ SE; $n = 6$ for high- and low-enrichment sites and $n = 10$ for moderate-enrichment sites. See text for the regression equations.

The strength of biomass–nutrient regressions in the northern Ozarks can be partially explained by the broad range of nutrient conditions in the streams we sampled (Table 1). TN and TP concentrations ranged over 1–3 orders of magnitude and regressions were strongly influenced by high biomass in sites classified as highly enriched. A similar nutrient range was present in the streams sampled by Aizaki and Sakamoto (1988). Another factor adding to the strength of our regressions is the use of long-term averages of TN and TP, which likely provide a more accurate measure of nutrient loading than single point or seasonal mean estimates (Biggs and Close 1989).

Intercorrelation between TN and TP ($r = 0.97$) prevented a determination of the relative influence of each nutrient. Nitrogen, however, is likely the more important of the two in northern Ozark streams. Except in highly enriched sites, mean annual TN was $<750 \mu\text{g}\cdot\text{L}^{-1}$, considerably lower than the range of summer means ($1110\text{--}2560 \mu\text{g}\cdot\text{L}^{-1}$) typical of streams in the

Ozark Plateau (Jones et al. 1984; Smart et al. 1985). TN and $\text{NO}_3\text{-N}$ concentrations in northern Ozark streams are particularly depressed during low-flow periods, and experimental enrichments conducted in one stream in the region have demonstrated that periphyton is N-limited (Lohman et al. 1991).

While floods were most common in spring and fall in northern Ozark streams, benthic Chl *a* maxima also occurred during these seasons. Sumner and Fisher (1979) noted that benthic Chl *a* maxima in Fort River, Massachusetts, coincided with the period before full canopy development in May and again in November after leaf fall, suggesting that shading may limit periphyton growth during summer. More intense grazing pressure may also suppress periphyton biomass during summer months in northern Ozark streams. Snail grazing is conspicuous in some streams in the region during summer (Lohman et al. 1991). Stoneroller minnows (*Camptostoma anomalum*), shown to be important grazers in prairie-margin streams (Power and Matthews 1983), are also ubiquitous throughout the northern Ozarks (Pflieger 1975), but we do not know if they ever limit periphyton biomass in these streams or if their impact is greater in summer than in other seasons.

Although floods periodically reduced periphyton biomass in all northern Ozark streams, recolonization was always most rapid at sites with high levels of nutrient enrichment. These temporal patterns indicate that while flood frequency and intensity are dominant factors, nutrients are influential in determining periphyton biomass during flood-free periods. Horner et al. (1983) have suggested that nuisance algal conditions occur when levels of periphytic Chl *a* exceed $100\text{--}150 \text{ mg}\cdot\text{m}^{-2}$. Chl *a* was greater than $150 \text{ mg}\cdot\text{m}^{-2}$ on 18.9% of the dates sampled in highly enriched streams, 5.3% of the time in moderately enriched streams, and only 0.4% at low-nutrient sites (Table 2). Notwithstanding floods, nutrient enrichment is a strong indication of potential nuisance algal conditions in northern Ozark streams.

Postflood recovery rates during fall 1986 were clearly influenced by ambient nutrient concentrations, and patterns of recolonization differed between sites of low enrichment and those

of high and moderate enrichment. Based on the growth curves fitted for each nutrient group, periphyton biomass would reach nuisance densities ($>150 \text{ mg} \cdot \text{m}^{-2}$) within 21 d after catastrophic flooding in highly enriched sites and within 28 d in moderately enriched sites, but average benthic Chl *a* would never exceed roughly $75 \text{ mg} \cdot \text{m}^{-2}$ in low-nutrient sites. Our analysis provides an approximation of maximum biomass that might be expected in northern Ozark streams during prolonged flood-free periods. Actual measurements of maximum biomass over 1985–86 at low-enrichment sites were greater than predicted but at five of six sites were $<150 \text{ mg} \cdot \text{m}^{-2}$ and were generally far below maximum levels measured at high- and moderately enriched sites (Table 2). These differences in periphyton accrual patterns suggest that nuisance conditions are unlikely to develop in northern Ozark streams with TN and TP concentrations less than those that occur in the sites classified as low nutrient. In contrast, nuisance algal conditions could be expected in nutrient-rich streams when flood-free periods exceed 3–4 wk, especially if such periods occur during spring or fall.

Our results support the hypothesis of Grimm and Fisher (1986) that nutrient availability strongly influences rates of periphyton accrual. Their model would also predict that, regardless of nutrient conditions, all sites should arrive at a similar maximum standing crop unless preempted by some other limiting factor. Patterns of periphyton growth in northern Ozark streams, however, do not support this conclusion. Differences in biomass among high-, moderate-, and low-nutrient groups during fall 1986 persisted after 42 d of colonization. Whereas biomass in low-nutrient sites reached a steady state after 28 d, periphyton continued to accrue for 42 d after disturbance in high- and moderately enriched sites.

Differences in maximum standing crops may reflect differences in algal community structure. Welch et al. (1988) found that diatom-dominated assemblages were not associated with high biomass whereas assemblages dominated by filamentous species could be expected when benthic Chl *a* exceeded $100 \text{ mg} \cdot \text{m}^{-2}$. Although we did no taxonomic analysis of algal communities in fall 1986, Baysinger-Daniel (1989) identified predominant taxa in the same study sites during fall 1984 and found that filamentous species (*Cladophora*, *Stigeoclonium*) were more common in sites that we have designated as high and moderately enriched whereas low-nutrient sites were dominated by diatom–blue-green assemblages. Thus, differences in community composition may help to explain differences in both the shape and magnitude of periphytic growth patterns among streams with various nutrient regimes.

Our results demonstrate that periphyton biomass and the development of nuisance algal mats are strongly associated with nutrient enrichment in northern Ozark streams. Establishing nutrient guidelines to control periphyton biomass in streams, however, is complicated by the interacting effect of flood disturbance. As pointed out by Welch et al. (1989), the use of empirical correlation to determine the critical nutrient concentrations that will limit algal biomass in streams may not always be reliable. Nevertheless, patterns of periphyton biomass in northern Ozark streams indicate that it is possible to determine which streams are most likely to develop high periphyton biomass when hydrologic conditions are favorable for growth.

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Phosphate and Iron Limitation of Phytoplankton Biomass in Lake Tahoe

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Field measurements and bioassay experiments were coupled to investigate the interdependent processes affecting phytoplankton biomass at Lake Tahoe using a trace metal protocol. Water samples were analyzed for suspended particulate matter, dissolved organic carbon, major ions and macronutrients, adenosine triphosphate, and phytoplankton abundance. Concentrations of total Cd (≤ 18 pM), Cu (2.25–8.85 nM), and Fe (22–49 nM) were similar to or lower than those reported for other oligotrophic lakes. Bioassays were carried out to assess the response of inoculated, single-species diatom populations (*Cyclotella meneghiniana* and *Aulocosiera italica*) to additions of synthetic chelators (EDTA, EDDHA) and phosphate. A chemical speciation model along with the field data was also used to predict how trace metal speciation, and hence bioavailability, was affected by the chelator additions. Results suggest that phosphate was limiting to phytoplankton biomass. Other solutes, Fe in particular, may also exert controls on biomass. Nitrate limitation seems less likely, although Fe-limiting conditions, as suggested by the bioassays, may have led to an effective N limitation because algae require Fe to carry out nitrate reduction. Small perturbations in water chemistry may have pronounced effects on phytoplankton biomass in oligotrophic systems where essential nutrients are at low concentrations.

Des mesures sur le terrain ont été effectuées en parallèle avec des essais biologiques suivant un protocole orienté sur les oligo-éléments, en vue d'étudier les processus d'interdépendance touchant la biomasse phytoplanctonique dans le lac Tahoe. Dans des échantillons d'eau, on a analysé la concentration des particules en suspension, du carbone organique dissous, des principaux ions et des macro-éléments, de l'adénosine triphosphate et du phytoplancton. Les concentrations de Cd total (≤ 18 pM), de Cu total (2,25 à 8,85 nM) et de Fe total (22 à 49 nM) étaient équivalentes ou inférieures à celles que l'on a enregistrées dans d'autres lacs oligotrophes. Des bio-essais ont été effectués afin d'évaluer la réaction de populations inoculées de diatomées à espèce unique (*Cyclotella meneghiniana* et *Aulocosiera italica*), auxquelles on a ajouté des agents chélateurs synthétiques (EDTA, EDDHA) et des phosphates. On a également utilisé un modèle de spéciation chimique ainsi que des données prises sur le terrain en vue de déterminer quelle incidence l'addition d'agents chélateurs a eue sur la spéciation en fonction des oligo-éléments, et donc sur la biodisponibilité. Les résultats semblent signifier que les phosphates réduisent la biomasse phytoplanctonique. D'autres solutés, le Fe en particulier, peuvent également entraîner une réduction de la biomasse. Toutefois, la réduction due aux nitrates semble moins probable, malgré que les conditions de limitation due au Fe auraient pu entraîner une réduction réelle du N, comme semblent l'indiquer les bio-essais, parce que la réduction des nitrates par les algues ne se fait qu'en présence de Fe. De faibles perturbations dans la composition chimique de l'eau peuvent avoir de grandes répercussions sur la biomasse phytoplanctonique dans les réseaux oligotrophes à faible concentration de matières nutritives essentielles.

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Oligotrophic Lake Tahoe has been famous for its water clarity since Le Conte in 1873 first recorded a Secchi disk transparency of 33 m (Le Conte 1883). However, since 1958, Secchi disk transparency has declined at an average rate of $0.37 \text{ m} \cdot \text{yr}^{-1}$ (Goldman 1988), and in 1988 the average Secchi disk transparency measured near midlake was approximately 25 m (Byron et al. 1988). Coincident with euphotic zone compression, the average rate of primary productivity has increased by $5.6\% \cdot \text{yr}^{-1}$ and has more than doubled since 1959 (Goldman 1981).

Definitive identification of the processes responsible for these changes has been difficult in this oligotrophic system. Over the last 30 yr, numerous ^{14}C bioassay studies have been used to measure the response of the Lake Tahoe phytoplankton community to various nutrients, chelators, and trace metal combinations. Historically, results of these bioassays have generally been interpreted as evidence of nitrate limitation. Decreases in water clarity and increases in primary productivity have been

attributed to increases in nitrate concentration (Goldman 1964, 1972, 1981), although Lean and Pick (1981) argued that interpretation of an early study (Goldman 1964) was problematic, due to premature sampling of the bioassays and possible damage to the photosynthetic apparatus. They also asserted that conclusions from a subsequent investigation (Goldman 1972), in which short-term incubations were stimulated by the combined addition of nitrate and phosphate but not by nitrate alone, were also tenuous because these bioassays were carried out in November, when nitrate would be brought to the euphotic zone by deep mixing. Lean and Pick (1981) further pointed out that no mention was made of the fact that the addition of nitrate alone caused the greatest depression in ^{14}C uptake. Finally, because no precautions were taken to avoid trace metal contamination in these and subsequent studies, the strength of these results are weakened.

Goldman (1988) observed a significant ($r^2 = 0.36$, $P < 0.018$) increase in nitrate concentration ($\text{NO}_3\text{-N}$) over the period

1973–86 and also a significant positive relationship ($r^2 = 0.23$, $P < 0.05$) between $\text{NO}_3\text{-N}$ and carbon fixation for that same period. Currently, $\text{NO}_3\text{-N}$ and ammonium ($\text{NH}_4\text{-N}$) concentrations are low, rarely exceeding $0.50\ \mu\text{M}$ in the euphotic zone, except immediately following complete lake mixing. Whereas $\text{NO}_3\text{-N}$ concentrations increase with depth and can reach $2.84\ \mu\text{M}$ towards the lake bottom, NH_4 concentrations remain low throughout the water column (Goldman 1988).

Although it is now suspected that Lake Tahoe phytoplankton may be more phosphate limited than it was two decades ago and that trace metals may also be limiting (Goldman 1964; Arneson 1979; Goldman 1981; Byron et al. 1983; Lane and Goldman 1984; Byron and Goldman 1988; Goldman 1988), evidence of this shift has not been rigorous. Postulated evidence for phosphate or trace metal limitation has included increasing N:P ratios and the response of ^{14}C uptake over 4- to 6-d incubations with the addition of streamwater, nutrient, chelator, and trace metals. None of these studies, however, has employed rigorous techniques for the analysis of trace metals, nor have precautions necessary to avoid inadvertent trace metal contamination or loss been adhered to which are especially critical for investigations of oligotrophic systems (Fitzwater et al. 1982; Marra and Heinemann 1987). Moreover, none of these studies considered trace metal speciation.

Finally, there has been great year-to-year variability in total P, which may obscure any long-term temporal trends (Goldman 1988). This apparent high year-to-year variability seems likely to be due to the use of only a single sampling station to characterize a spatially heterogeneous system combined with estimation errors resulting from the very low P concentrations. Presently, spring and summer total P concentrations in Lake Tahoe range from 6×10^{-2} to $20 \times 10^{-2}\ \mu\text{M}$; dissolved concentrations range from below the limit of detection ($6.4 \times 10^{-2}\ \mu\text{M}$) to $10 \times 10^{-2}\ \mu\text{M}$, making it difficult to resolve the structure of the P profile with depth.

The objective of our study was to investigate the possibility that phytoplankton biomass in Lake Tahoe is currently phosphate limited with secondary effects caused by the availability of trace metals. There were two components to this study: (a) a description of the vertical distribution (five depths) of temperature, and concentrations of trace metals, suspended particulate matter (SPM), macronutrients, adenosine triphosphate (ATP), dissolved organic carbon (DOC), major ions, and direct cell counts (acridine orange) were determined at a deep midlake and a shallow nearshore station "Index" and (b) experimental bioassays.

The purpose of the descriptive component was to (1) determine how the chemical variables and phytoplankton biomass (measured by ATP) might differ between midlake and nearshore areas, (2) provide initial information on trace metal concentrations in the water column to aid in the interpretation of bioassay results, and (3) determine the chemical speciation of some of the biologically important trace metals in the water.

The bioassays were designed to examine the responses of algal monocultures grown in filtered lake water to changes in phosphate concentrations and trace metal speciation, and hence bioavailability. Changes in trace metal speciation were affected by the addition of the chelator ethylenediaminedihydroxyphenylacetic acid (EDDHA) or ethylenediaminetetraacetic acid (EDTA).

Study Site

Lake Tahoe (altitude 1879 m) is a deep oligotrophic lake (Z_{max} 501 m, $\bar{Z}$ 313 m). Lake surface area ($500\ \text{km}^2$) is large

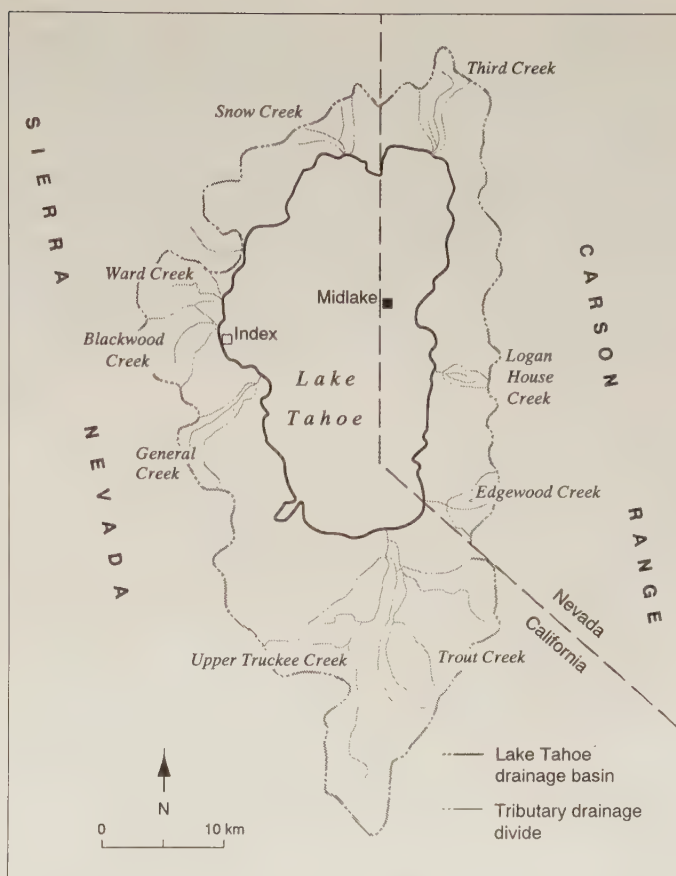


FIG. 1. Map of study site showing Index and Midlake stations.

relative to the watershed ($800\ \text{km}^2$), which is composed primarily of granitic and volcanic formations (Fig. 1). Lake Tahoe never freezes, and its waters are oxic to lake bottom, with dissolved year-round oxygen concentrations of approximately $11\ \text{mg}\cdot\text{L}^{-1}$ (Holm-Hansen et al. 1976). There is one outlet, the Truckee River, where release is controlled by a dam. The lake volume is $156\ \text{km}^3$, with a retention time of approximately 700 yr. The lake does not turn over every year and does so only in response to winds during severe February or March storms. The nearshore station (Index) was located near Blackwood Creek, which is thought to be a major nutrient source for the lake (Axler et al. 1982). The midlake station was located on the California–Nevada border, west of Skunk Harbor (Fig. 1). These stations have been monitored by the Tahoe Research Group at the University of California, Davis on a routine basis (Axler et al. 1982).

Materials and Methods

Although bioassays with natural assemblages more accurately represent the true community at the beginning of an experiment, long-term experiments with oligotrophic communities often experience marked shifts in species composition. This shift in community structure could introduce an additional element of uncertainty when interpreting the results. Using a monoculture avoids the problem of species composition shifts and permits repeated experimentation with the same organism at the recognized expense of community representation.

In batch cultures, yields are based on initial nutrient concentrations, which during long-term experiments may decrease greatly, while other concentrations (e.g. DOC) may increase in a manner that is not representative of the system they are

trying to model. However, because physical variables such as light and temperature can be controlled in the laboratory, the interpretation of results for laboratory experiments may be less ambiguous than for studies whose data rely on field measurements alone.

Mindful of the strengths and weaknesses of the above approaches, in this study we integrated information from field data and results of bioassays to increase our understanding of nutrient limitation at Lake Tahoe. We believe that the use of multiple complementary approaches can be an effective strategy, as field data can be used to verify and aid in interpretation of the hypothesis generated by the bioassays.

Water Chemistry

Water samples were collected during the second and third years of a drought: April 26 and May 17, 1988, and April 20, May 17, and September 27, 1989. Spring collections coincided with peak snowmelt runoff. Water from five depths at the Index (15, 50, 85, 140, and 180 m) and Midlake (15, 85, 180, 300, and 400 m) stations was collected and analyzed for cations (Na, K, Ca, and Mg), SPM, DOC, phytoplankton abundance, and trace metals. Data on $\text{NO}_3\text{-N}$ concentrations were provided by the Tahoe Research Group. A discrete water sample was taken at each depth at each station with Teflon-lined sample bottles (10-L Go-Flo¹ bottles, General Oceanics). The cleaning procedure for the sample bottles is described in the trace metal section below. Lake bottom depths at the Index and Midlake stations were 200 and 500 m, respectively. We did not attempt to measure dissolved orthophosphate concentrations, as they typically occur at or near the detection limit ($6.4 \times 10^{-2} \mu\text{M}$) (Tahoe Research Group).

Cations were analyzed by flame atomic absorption spectroscopy (Thermo Jarrell-Ash Vide and Perkin-Elmer). The coefficients of variation for these analyses (based on three determinations) were $\leq 5\%$ of the concentration and represented analytical error, as they are based on replicate subsamples from a single sample. Dissolved Si was spectrophotometrically determined by the formation of the yellow molybdosilicate complex: the detection limit was $7.76 \mu\text{M}$ (APHA 1971). DOC was measured on a total carbon analyzer (Dohrmann) by persulfate and ultraviolet oxidation ($n = 3$ for each of three injections). The coefficient of variation averaged 15% and never exceeded 25%. Detection limits were 0.25 ppm as governed by the instrument. SPM was measured by filtering 50 mL of sample through a precombusted (400°C , 3 h) 25-mm-diameter GF F Whatman glass fiber filter (0.7- μm nominal pore size) or through a Nuclepore polycarbonate membrane (0.4- μm pore size). To minimize filter fragmentation, glass fiber filters were placed on precombusted, preweighed aluminum foil trays and left on the trays for subsequent weighings. After filtration, both types of filters were dried in a desiccator and weighed. The GF F filters were combusted again in a muffle furnace (400°C , 3 h) and weighed in order to obtain ashed weights. Three filters of each type were prepared per sample.

Numerous papers have shown how contamination can greatly affect the outcome of studies focusing on nutrient limitation (Fitzwater et al. 1982; Marra et al. 1981). Accordingly, stringent precautions were taken throughout the investigation to avoid trace metal contamination. Clean techniques were adhered to while collecting the water samples, processing trace metal samples, and for bioassays (Fitzwater et al. 1982). Water

TABLE 1. Fixed parameters used in the HYDRAQL program for determining chemical speciation in bioassays. Values used in the program are based on observed values.

Parameter	Program value	Observed value
pH	7.6	7.2–7.8
DOC	0.5 ppm	0.4–0.9 ppm*
Cd^{2+}	$1.3 \times 10^{-11} \text{ M}$	— ^b
Cu^{2+}	$6 \times 10^{-9} \text{ M}$	See Table 2
Fe^{3+}	$4 \times 10^{-8} \text{ M}$	See Table 2
Zn^{2+}	$2 \times 10^{-11} \text{ M}$	— ^c
NO_3^-	$4 \times 10^{-7} \text{ M}$	— ^d
PO_4^{3-}	$2 \times 10^{-7} \text{ M}$	— ^d
Ca^{2+}	$2 \times 10^{-4} \text{ M}$	See Table 4
Mg^{2+}	$1 \times 10^{-4} \text{ M}$	See Table 4
K^+	$2.7 \times 10^{-4} \text{ M}$	See Table 4
CO_3^{2-}	$9 \times 10^{-4} \text{ M}$	See Table 4
SO_4^{2-}	$1.5 \times 10^{-5} \text{ M}$	See Table 4
Cl^-	$4.2 \times 10^{-5} \text{ M}$	See Table 4
SiO_3^{2-}	$4.4 \times 10^{-4} \text{ M}$	See Table 4

*We assumed a conversion of $5 \times 10^{-7} \text{ M}$ of binding sites per mg C of the dissolved organic material (Mantoura and Riley 1975; McKnight et al. 1983).

^bBased on this study; data not shown.

^cBased on surface measurements.

^dBased on values reported by the Tahoe Research Group.

samples were collected with Teflon-lined sample collection bottles suspended from a nonmetallic (Kevlar) line. An inverted plastic funnel was used to minimize atmospheric contamination during sample collection. Samples were acidified to pH 1.8 with redistilled nitric acid (Seastar Chemicals) in a class 100 laminar flow hood. Because SPM concentrations were low ($0.44\text{--}0.85 \text{ mg}\cdot\text{L}^{-1}$) and to minimize contamination due to sample transfer, analysis for trace metals was done on unfiltered samples. Trace metal concentrations therefore reflect total concentrations. To determine whether there were pronounced differences between unfiltered and filtered samples, samples from 15 m were also filtered through a 47-mm Nuclepore polycarbonate membrane filter (0.2- μm pore size). The filters were cleaned by soaking in 11 N HNO_3 for 2 d and rinsing in deionized water. Samples were filtered in the laminar hood through a FEP filtration funnel (Savillex) into HDPE bottles.

Cd samples were concentrated 50-fold by APDC/cobalt precipitation (Bloom and Crecelius 1984). Nitric acid (1%) was used to reconstitute the sample. Co was prepared by dissolving 5 g of cobalt acetate (J. T. Baker, Inc.) in 50 mL of hydrobromic acid, evaporating to dryness in a FEP beaker, and reconstituting in 650 mL of deionized water. The CoBr solution was then cleansed by anion exchange using an AGX1-X8 resin (Bio-Rad Laboratories) as described by Linn (1988). APDC was cleaned by extraction through a Chelex 10 resin (100–200 mesh, Bio-Rad).

Trace metals were analyzed by graphite furnace atomic absorption spectrophotometry with Zeeman background correction. After cleaning, all reagents were checked for Cd contamination prior to use. To ensure that neither the Co nor APDC caused signal suppression or enhancement, blanks and standards were prepared with varying concentrations of Co or APDC. The cleaned Co solution was then diluted to $3.4 \mu\text{M}$ with deionized water. Cu and Fe concentrations were determined by direct injection using standard additions. The detection limits for Cd and Fe were 17 pM and 7.5 nM, respectively. The

detection limit for Cd was 0.22 nM, based on two times the standard deviation of the blanks.

The water chemistry data were used in the chemical speciation program HYDRAQL (Papelis et al. 1988) to model how trace metal speciation in the bioassay experiments would be affected by the chelator additions (Table 1). Stability constants for metal-EDDHA and metal-EDTA reactions are those reported by Martell and Smith (1974), Anderegg (1977), and Perrin (1979).

Phytoplankton ATP and Counts

ATP was used as an indicator of phytoplankton biomass, rather than chlorophyll (Chl *a*), because it is a more reliable measure of live and heterotrophic cells and because negligible amounts of ATP are recovered from particulate matter (Holm-Hansen and Booth 1966). Furthermore, C:Chl ratios can range from 10 (Laws and Bannister 1980) to 250 or more (Holmes et al. 1967; Sakshang and Holm-Hansen 1977; Malone and Chervin 1979; Laws and Bannister 1980; Redalje and Laws 1981) and can vary as much as two- to fourfold in response to diurnal light variations (Sournia 1974; Eppeley et al. 1979), nutrients (Bannister and Laws 1980; Goldman 1980), species composition (Chan 1980), and physiological state and temperature (Eppeley and Renger 1972; Yoder 1979). ATP was extracted with cold (1–3°C) H_3PO_4 and preserved within 8 h of collection using a modification of a technique described by Karl and Holm-Hansen (1978). One litre of sample was filtered through either 47-mm-diameter, 0.45- μm Durapore (polyvinylidene difluoride, Millipore) or Asypore (cellulose acetate, Dominick Hunter) membranes after prescreening with a 60- μm screen to remove zooplankton. Prior testing of the filters demonstrated that they were nonreactive and gave excellent recoveries. Filters and residue were immersed for 5 min in 7 mL of ice-cold 0.15 M H_3PO_4 to extract the ATP. After extraction, 5 mL was pipetted into a polyethylene scintillation vial, and 1 mL of 0.1 M Tris buffer (pH 7.8) was added to the sample, which was then neutralized to pH 7.4 using 3.0 and 1.5 M NaOH. The sample was then quick-frozen in an acetone dry ice bath and stored at -70°C until analysis.

The night prior to ATP analysis, several vials of firefly lantern extract FLE-50 (Sigma) were each reconstituted with 5 mL of distilled water and 10 mL each of 0.04 M MgSO_4 and 0.1 M Na_2HAsO_4 at pH 7.4. The solutions were aged overnight in the dark and were combined immediately before analysis to yield a homogeneous mixture. The FLE-50 solution was filtered through an Asypore filter to remove undissolved luciferin. Standards were prepared in 0.02 M Tris (pH 7.5), buffered and adjusted to the same pH as were the samples. Thawed samples and freshly prepared standards were immersed in a boiling water bath for 2 min and then cooled to room temperature. Four hundred microlitres of luciferin was then injected into 250 μL of each sample. Samples were measured on a Turner TD 20-e luminimeter. Three replicates were analyzed from each vial, and three vials were prepared per water sample. The analytical detection limit was 5×10^{-10} M, which, when multiplied by the concentration factor, corresponded to 3.5 pM. The coefficient of variation for ATP and all other analyses was calculated from three subsamples of each sample and thus represents analytical error. For the ATP analyses the coefficient of variation was $\leq 3\%$.

As a second measure of phytoplankton biomass, direct microscope counts of phytoplankton were also made for the September 1989 samples. Acridine orange counts were done in order to include picoplankton which comprise a significant por-

portion of the phytoplankton population in Lake Tahoe, especially in the deep waters. Samples were preserved in 2% formaldehyde for microscope counts. One millilitre of 0.1% acridine orange was used to stain 10 mL of sample. The sample was filtered through a black 25-mm, 0.2- μm Nuclepore filter and counted on a Leitz Dialux 20 microscope with a 480-nm excitation filter. The microscope optics were the same as those described by Harvey (1987). For each filter, counting was carried out until 200 individuals had been observed, and in no case were less than five fields counted. Phytoplankton of each of three filters were counted per sample. The average coefficient of variation was $<20\%$ of the mean.

Bioassays

Bioassays examined the response of phytoplankton biomass to additions of phosphate and the chelators EDTA and EDDHA. P was added as K_2HPO_4 at concentrations to span and slightly exceed the observed range for P in Lake Tahoe. Chelator was added at concentrations sufficient to significantly complex the trace metals. A factorial design in 1988 had three levels of P addition (0.0, 0.2, and 0.4 μM) and three levels of EDTA (0.0, 0.2, and 0.4 μM) added to the filtered water. EDTA altered the manner in which trace metals were chelated or held in solution, which in turn affected their availability to algae. The design of the second series was similar to that of the first, except that EDDHA replaced EDTA. EDDHA, a particularly strong Fe chelator, was initially developed as a fertilizer additive to improve Fe solubility in alkaline soils (Holmes and Brown 1955) and has been used in algal growth media formulations (Anderson and Morel 1982; Kuwabara 1985).

All treatments were replicated in triplicate. Owing to culturing space constraints, the EDTA experiments were carried out during April of both years and the EDDHA treatments were carried out in May.

Comparisons of results between controls for each water sampling date provided an indication of the effect of sampling date. For most bioassays, water from the Index station (15 m) was used, but in 1989, water from the Midlake station (15 m) was also used for the EDDHA bioassays to permit comparison between the two stations. In this design, two levels of EDDHA (0.0 and 0.4 μM) were crossed with two levels of P addition (0.0 and 0.4 μM) to Index station water. For the bioassays with Midlake water, an additional fifth treatment was used, with 0.2 μM EDDHA and 0.2 μM added P.

To obtain phytoplankton-free lake water for use in the bioassays, lake water was vacuum-filtered (≤ 10 psi (1 psi = 6.895 kPa)) through 0.2- μm polycarbonate filters using a FEP (Savillex) filtration funnel into high-density polyethylene bottles (HDPE). Filtering was done in a class 100 laminar flow hood and the water later dispensed into polycarbonate Erlenmeyer flasks for culturing. Bottles and filtration flasks were washed by a procedure similar to that used for the trace metal protocol, except that HCl instead of HNO_3 was used.

Bioassays were conducted with monocultures of two diatoms. Because we were initially unable to obtain a Lake Tahoe isolate of *Cyclotella* sp., the diatom *Cyclotella meneghiniana* (Czarnecki 1987) was used for the 1988 bioassays. In 1989, a Lake Tahoe isolate of *Aulacoseira italica* (formerly *Melosira italica*), a representative diatom of Lake Tahoe, was used for comparison with the *Cyclotella* results. The algae were first grown in modified S-3 medium formulated to have low nutrient and trace metal concentrations (Kuwabara and North 1980; Kuwabara 1985), similar to those that might be encountered in Lake Tahoe water. Culturing protocol was similar to that of

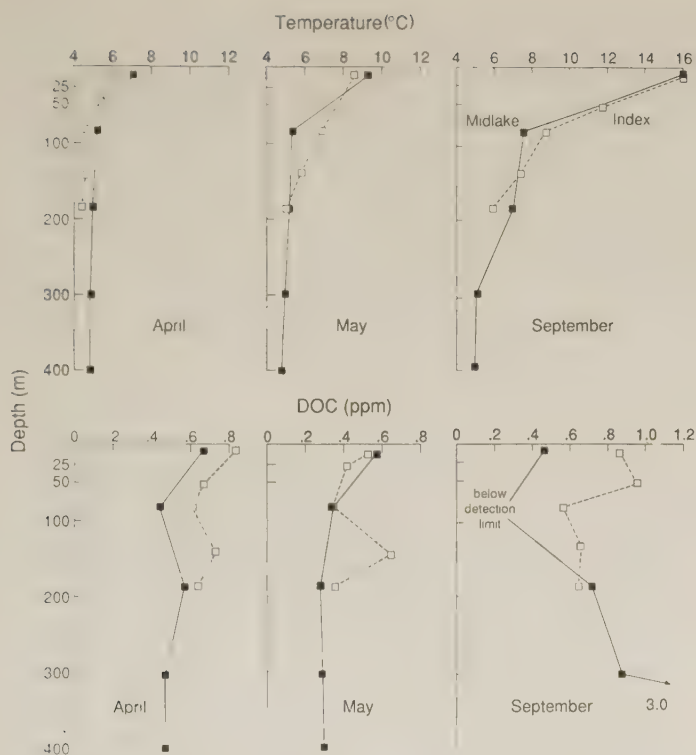


FIG. 2. Temperature and DOC concentrations for the three sampling dates in 1989. The average CV is 15% and is based on three subsamples and represents analytical error. Solid squares indicate Midlake samples; open squares indicate Index station samples.

Kuwabara (1985), except that the major element concentrations (see Table 4) were modified to simulate the ionic strength of the lake water. Algae were inoculated in 50 mL of filtered lake water at a concentration of 2000 cells·mL⁻¹.

Response was measured as dry weight per millilitre at the time when maximal cell concentration was achieved; this usually occurred within 14 d after inoculation. Algal cultures were monitored with an electronic particle counter (model No. ZBI, Coulter Electronics) every 3 d at the beginning of the experiment and then daily as cell concentrations increased. Dry weight density (milligrams per litre) was calculated as the product of numerical cell density, mean cell volume, and cell density (Miller et al. 1978). The cultures were grown at 7°C under cool white fluorescent lights at 65 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ on a 12 h : 12 h diurnal cycle.

Results

Temperature

Midlake station surface temperature (15 m) was slightly warmer than that at the Index station in April and May (Fig. 2). By September, the surface water temperature was similar at the two stations. Surface water temperature increased at both stations on each subsequent sampling date.

Water Chemistry

Although Cu (2.10–8.85 nM) and Fe (21.84–48.69 nM) concentrations were not uniform throughout the water column, concentrations varied no more than four- and twofold, respectively (Table 2). Concentrations of total Cu and Fe were higher at 15 m than at greater depths and tended to increase near the lake bottom. No systematic differences were detected between

TABLE 2. Mean trace metal concentrations (nM, CV in parentheses) in unfiltered and filtered (*) water samples collected in September 1989. Filtered samples were passed through a 0.2- μm pore size Nuclepore filter. CV are calculated from three subsamples and represent analytical error.

Depth (m)	Cu		Fe	
	Index station	Midlake station	Index station	Midlake station
15	6.90 (1)	8.85 (17)	43.69 (3)	
15*	6.89 (2)		35.80 (3)	
50	1.95 (1)		31.69 (8)	
85		2.40 (18)	21.84 (18)	22.76 (12)
140	2.25 (3)		27.20 (3)	
180	3.15 (3)		30.78 (15)	22.02 (7)
300		2.10 (2)		39.56 (12)
400		3.75 (1)		48.69 (3)

TABLE 3. Mean SPM concentrations (mg·L⁻¹, CV in parentheses) on May 17, 1989. Samples were passed through Whatman GF/F (0.7- μm pore size) and Nuclepore (0.45- μm pore size) filters. CV are calculated from three subsamples and represent analytical error.

	GFF			
Depth (m)	Dry wt	Ashed wt	% organic matter	Nucleopore dry wt
Index station				
15	0.85 (11)	0.24 (56)	72	0.44 (39)
50	0.50 (31)	0.37 (28)	26	0.16 (8)
85	0.56 (9)	0.17 (30)	70	0.23 (1)
140	0.43 (16)	0.19 (36)	56	0.17 (2)
180	0.50 (7)	0.08 (86)	84	0.19 (18)
Midlake station				
15	0.53 (0.9)			0.42 (1)
85	0.47 (7)			0.34 (5)
180	0.40 (2)	0.17 (40)	58	0.23 (52)
300	0.19 (36)	0.05 (173)	74	0.20 (8)
400	0.27 (6)	0.08 (367)	71	0.23 (1)

stations. Fe concentrations were an order of magnitude higher than Cu concentrations (Table 2). At the Index station, Fe and Cu profiles were similar, with the highest concentrations at 15 m. Fe and Cu concentrations at both stations increased towards the bottom. Comparison of total and dissolved Fe and Cu concentrations at the 15-m Index station indicated that these metals were primarily partitioned into the dissolved phase (i.e. in the <0.2 μm filtrate). Cd concentrations were near or below the detection limit of 17.7 pM throughout the water column, except at 15 m, where concentrations were slightly higher (20 pM). These concentrations are less than or similar to those reported for other oligotrophic lakes (Holbrook et al. 1985; Murray 1987).

DOC concentrations were mostly in the range of 0.3–1.0 ppm, with a maximum of 3 ppm observed at 400 m in September (Fig. 2). In April and September, although not in May, DOC concentrations in the top 200 m of the lake tended to be consistently greater at the Index than at the Midlake station. Although vertical profiles were sometimes erratic with depth, there was a general tendency for DOC values to decrease with depth in the first 200 m and then remain constant (April, May) or increase (September) with depth below 200 m.

SPM was most abundant in the upper water column and, on the basis of ashed weight recoveries, was composed primarily

TABLE 4. Major ion concentrations (10^{-4} M) at Midlake and Index stations from April samples. Results of Mariner et al. (1977) and Friedman et al. (1964) are presented for comparison. CV is 5% of concentration and represents analytical error.

Station	Depth (m)	HCO ₃ ^a	Cl	SO ₄	Na	K	Ca	Mg	Si	pH	Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)
Midlake	85	9.18	0.57	0.21	2.61	0.41	2.10	1.03	3.68	7.3	25
	300	8.68	0.42	0.21	2.57	0.41	2.15	1.03	3.75	6.8	24
Index	50	8.68	<0.28	<0.10	2.21	0.43	1.95	0.91	4.18	7.8	25
	140	9.02	0.57	0.16	2.57	0.41	2.15	0.99	4.43	7.8	25
Crystal Bay (Mariner et al. 1974)		8.30	0.67	<0.10	2.88	0.46	2.09	0.99	4.60	8.1	
Truckee Dam (Friedman et al. 1964)		8.40	0.62	0.25	2.70	0.46	2.24	1.80	4.90		

^aTotal alkalinity as HCO₃.

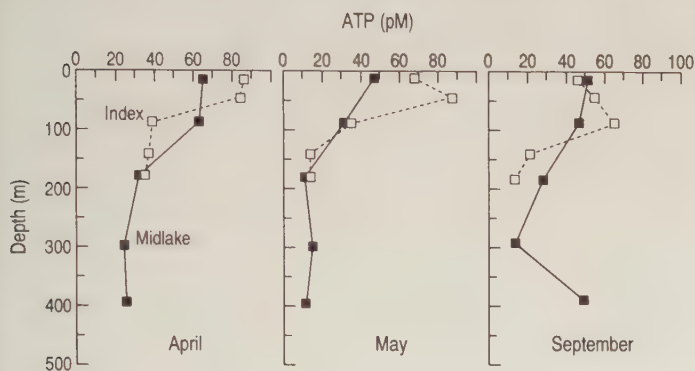


FIG. 3. ATP concentrations for the 1989 sampling dates. CV $\leq$ 3% is based on three subsamples and represents analytical error. Solid squares indicate Midlake station samples; open squares indicate Index station samples.

of organic matter (Table 3). SPM concentrations were not especially different between the two stations. However, at the Index station, SPM yields obtained with the GF/F filters were consistently two to three times those obtained with Nuclepore filters, while at the Midlake station, results with GF/F filters were similar to those obtained with the Nuclepore filters. The low SPM concentrations suggest that particulate-bound nutrients and trace metals may likewise be low. Major ion concentrations with the exception of Mg showed little variation with station or depth and were similar to those reported by Mariner et al. (1977) and Friedman et al. (1964) (Table 4), indicating minimal long-term changes in major ion chemistry.

Phytoplankton ATP and Counts

ATP concentrations generally decreased with depth (Fig. 3). The maximum ATP concentration at the Index station was observed at progressively greater depths as the season progressed. In spring (snowmelt period), ATP values in the upper water column were markedly higher at the Index station, near the mouth of Blackwood Creek, than at the Midlake station, but by September there was little difference between the stations. ATP profiles were more regular at the Midlake station, and tended to decline with depth whereas at the Index station, concentrations were more variable. The high concentration (47 pM) at 400 m at the Midlake station in September was coincident with the very high DOC concentration observed for the same sample (Fig. 2).

Direct counts of the phytoplankton, most of which were picoplankton, were made for the September samples (Fig. 4). Phytoplankton profiles at both stations during September resembled

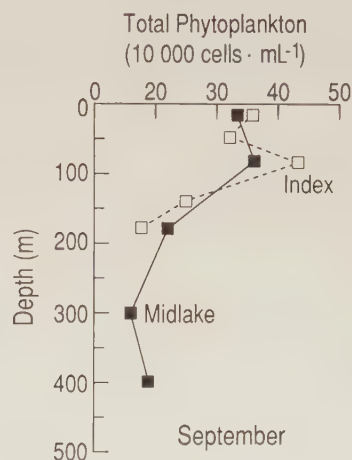


FIG. 4. Total phytoplankton concentrations for September 27, 1989. CV $\leq$ 20% is based on three subsamples and represents analytical error. Solid squares indicate Midlake station samples; open squares indicate Index station samples.

the ATP profiles. Only at 50 m at the Index station and 75 m at the Midlake station did ATP concentrations not increase when phytoplankton counts increased.

Bioassays

In the EDTA bioassays, the addition of $0.2 \mu\text{M}$ PO₄ increased biomass yield regardless of the level of EDTA (Fig. 5A and 5B). In 1988 with *C. meneghiniana* the average increase in yield from the zero P to $0.2 \mu\text{M}$ P treatments was 112%. In 1989, the average increase in yield of *A. italica* caused by addition of $0.2 \mu\text{M}$ P was 242%. In both cases, however, biomass yields at $0.4 \mu\text{M}$ added P were not detectably greater than those at $0.2 \mu\text{M}$ P. These results suggest that phosphate was initially the limiting factor and that upon further addition, some other factor or factors became important.

EDTA addition caused a marked increase in the biomass yield of *C. meneghiniana* in 1988. Its effect on *A. italica*, the Lake Tahoe isolate, in 1989 was less clear. Biomass yield for *A. italica* was highest for the no P added and $0.2 \mu\text{M}$ EDTA additions. However, the magnitude of the EDTA effect, at $0.4 \mu\text{M}$ P, was not as great in 1988 (Fig. 5A and 5B). As indicated by the approximately parallel nature of the three EDTA response curves, there was little indication of any interactive effect between P and EDTA in 1988 or 1989. Possibly, however, the EDTA effect in 1988 was greater at $0.4 \mu\text{M}$ P than at the lower P concentrations. These results suggested that EDTA may be ameliorating a toxic trace metal effect or facil-

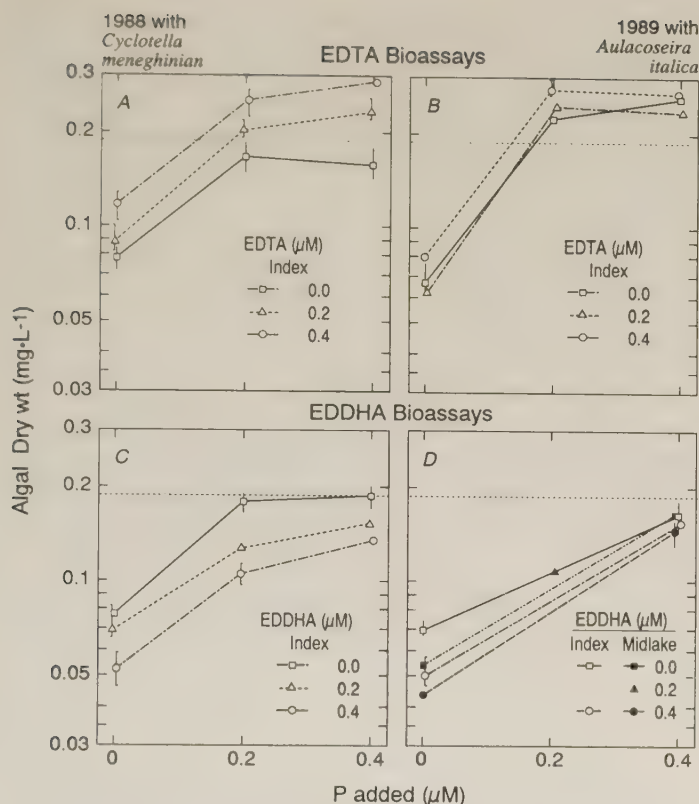


FIG. 5. Maximum algal biomass density (after ~2 wk) following addition of phosphate and chelator (EDTA or EDDHA) to algal monocultures. Error bars represent 95% CI and are shown when large enough to be seen in the figure. Nonoverlap of CI indicates a statistically significant difference ($P < 0.05$) for two means. The horizontal broken line is drawn to facilitate comparisons of maximum biomass yield between the treatments.

itating the availability of an essential nutrient. There was no indication that sampling time had an effect on biomass yield, as there was no significant difference in yield between the control treatments (no chelator, zero P addition) for the EDTA and EDDHA experiments which were carried out with water collected 3 wk apart.

Results of the EDDHA treatments also showed a strong positive increase in biomass yield as a result of P additions (Fig. 5C and 5D). The nature of the P effect observed with *C. meneghiniana* seemed partly a function of EDDHA concentration in that with no EDDHA addition, the biomass yield did not increase further as P was increased from 0.2 to 0.4 μM whereas it did increase at the two higher levels of EDDHA. Nevertheless, the biomass yield at 0.4 μM added P averaged 117% greater than at the zero P addition, with little variation (95–133%) among the three EDDHA levels.

The response of *A. italica* to P addition was somewhat stronger than for *C. meneghiniana*, with the biomass yield at 0.4 μM P averaging 205% greater than at no added P. There was relatively little variation (144–247%) in the magnitude of this effect over the range of EDDHA levels used as indicated by the approximately parallel nature of the response curves. Biomass yields with no P addition tended to be slightly higher in water from the Index station. Otherwise, results obtained for the two sampling sites were very similar.

Addition of EDDHA, a particularly strong Fe chelator, generally decreased biomass yields (Fig. 5C and 5D), in contrast with the effect of EDTA (Fig. 5A). The percent decreases

caused by EDDHA were generally greater for *C. meneghiniana* in 1988 than for *A. italica* in 1989.

Discussion

Phosphate Limitation

Addition of 0.2 μM P consistently stimulated an increase in biomass, especially by the lake isolate, a result that suggests P limitation. This response was consistent between years and stations, regardless of the algal species used. Significant differences in yield between EDTA experiments in 1988 and 1989 may have been due to the change in test species or changes in the chemical composition of the lake water when the lake completely turned over in February 1989 for the first time since 1985. In the 1989 EDDHA experiment, biomass yields for *A. italica* were slightly higher when grown in Index station water (although not always statistically significant), and April and May ATP concentrations were higher at the Index station (Fig. 3). This is consistent with the hypothesis that nutrient concentrations were higher at the Index station than at the Midlake station.

Long-term trends in lake conditions also support our conclusion that the algae biomass and presumably growth are P limited. While there has been a significant increase in $\text{NO}_3\text{-N}$ with time, the concentration of dissolved phosphate is low ($\leq 10 \times 10^{-2} \mu\text{M}$), and total P has shown extremely high year-to-year variability with no apparent temporal trend (Goldman 1988). Furthermore, the TN:TP molar ratio at Lake Tahoe is approximately 54 (Hunter et al. 1990) which, compared with the Redfield N:P ratio of 16, suggests P limitation.

However, it is now widely accepted that certain fractions of the N pool are more biologically available than others, and furthermore that N availability varies between phytoplankton and microheterotrophs (Wheeler and Kirchman 1986; Jorgensen 1987; Probyn 1988; Suttle and Harrison 1988). NO_3 and NH_4 are generally the preferred forms of N, although recent evidence indicates that certain fractions of the organic N pool are utilized by microheterotrophs (Jorgensen 1987; Wheeler and Kirchman 1986). Consequently, it has been alternatively suggested that the ratio of inorganic N : TP, rather than TN:TP, more accurately predicts nutrient limitation (Morris and Lewis 1988). When nutrient addition treatments at eight Colorado mountain lakes were indexed, 52 of 56 treatments had inorganic N : TP ratios >1 and were categorized as P limited or colimited with N. At Lake Tahoe, the inorganic N : TP ratio typically ranges from 6 to 12 (Tahoe Research Group), consistent with P limitation.

Fe Limitation and Chelator Effects

The failure of biomass yields to increase when orthophosphate additions were increased from 0.2 to 0.4 μM and the fact that the chelators had contrasting effects on biomass yield suggest that other factors may have limited biomass once P limitation had been removed. The two chelators differ in their ability to complex Fe(III). The log of the binding constant for Fe–EDTA is smaller than that for Fe–EDDHA by at least six orders of magnitude (Martell and Smith 1974; Anderegg 1977; Perrin 1979). Thus, one possible explanation for the results is that EDDHA reduced the availability of Fe to the algal cells and imposed Fe-limiting conditions in the bioassays. The stronger effects of both chelators in 1988 than in 1989 suggest that *A. italica*, the lake isolate, may have a lower Fe require-

ment than does *C. meneghiniana*, another observation consistent with potential Fe-limiting conditions.

The hypothesis of Fe limitation was explored with metal speciation calculations using the computer program HYDRAQL (Papelis et al. 1988). In the absence of chelator, the program predicted that >99% of the Fe and Cu was complexed by dissolved organic matter. The prediction that Fe and Cu would exist primarily in the dissolved phase is supported by trace metal data for the 15-m Index station, which show similar concentrations for filtered and unfiltered samples (Table 2). Approximately 80% of the Zn was uncomplexed, and the remaining Zn was inorganically complexed. Cd was primarily in the uncomplexed form. Calculations indicated that >99% of the Cu and Fe was still complexed upon the addition of 0.2 μM EDTA, but that complexation was now shared by EDTA (50% of the Fe and virtually all of the Cu). Increasing the EDTA concentration increased the proportion of Fe complexed with EDTA (~70%), the remainder being complexed with DOC.

For the 0.2- μM EDDHA treatments, speciation calculations indicated that >99% of the Cu and Zn was complexed by EDDHA. Cd complexation was divided between EDDHA (45%) and dissolved natural organic matter. Virtually all of the Fe was complexed by EDDHA, as was intended, thus decreasing the free Fe concentration relative to that in the EDTA treatments by six orders of magnitude. Dissociation of the Fe(III)-EDTA complex and subsequent release of Fe(II) is photolytically enhanced (Anderson and Morel 1982). In contrast, the Fe-EDDHA complex is very stable and does not undergo photodegradation. Thus, it is unlikely that Fe-EDDHA was available to the algae. Therefore, Fe-limiting conditions may have existed in the EDDHA treatments as a result of the lower free Fe concentration and the unavailability of EDDHA bound to Fe. Furthermore, because our estimate of Fe concentration (4×10^{-8} M, estimated from the 15-m Index station, the depth from which the bioassay water was taken) used to predict Fe speciation is high relative to Fe concentrations as a whole (Table 2) and higher than subsequent field measurements in 1990 (data not shown), it seems likely that Fe limitation may be more acute than postulated in this study.

In the EDTA bioassays, the addition of EDTA increased biomass yield. This may have been the result of Fe-EDTA being more available to the algae than Fe bound to naturally occurring dissolved organic matter. An alternative explanation is that EDTA alleviated a toxic response to Cu or Zn. Whether the concentration of Cu^{2+} and Zn^{2+} in the lake is toxic to the phytoplankton is uncertain. The estimated concentration of Cu^{2+} in the lake ($\sim 10^{-11}$ M) is much lower than the concentration ($\sim 10^{-8.8}$ M) at which toxic effects on the freshwater algae are first reported to appear (Sunda and Lewis 1978; Petersen 1982). Zn^{2+} in Lake Tahoe was computed to be $\sim 10^{-9}$ M. This is lower than the concentration found detrimental (7.5×10^{-8} M) to the chlorophyte *Selenastrum capricornutum* in a P-limited system (Kuwabara 1985). Because the calculated concentrations of Cu^{2+} and Zn^{2+} are at least an order of magnitude below those reported to be toxic, Cu^{2+} and Zn^{2+} toxicity seems unlikely, although community structural changes from highly metal-sensitive species may be possible. It is more likely that the increase in biomass yield after EDTA treatment occurred as a result of Fe-EDTA being more available than Fe bound to natural dissolved organic matter. Although Fe requirements for freshwater algae are not well defined, the concentrations we observed are similar to those that impose Fe deficiency in laboratory cultures (Anderson and Morel 1982).

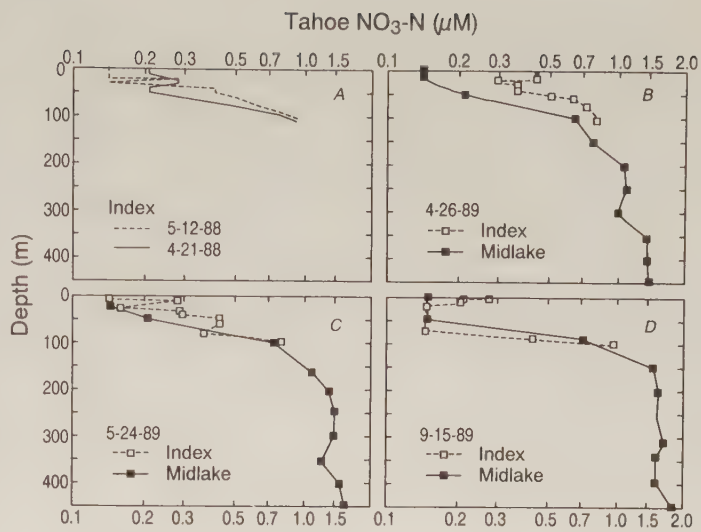


FIG. 6. $\text{NO}_3\text{-N}$ concentrations in Lake Tahoe for four sampling dates coincide with the times of our own sample collection. Dissolved P concentrations are near the detection limit (detection limit of 6.4×10^{-2} μM) and are too low to be accurately shown. Data provided by the Tahoe Research Group at the University of California, Davis.

As previously mentioned, it has been suspected that Lake Tahoe phytoplankton are phosphate and trace metal limited. However, evidence of this limitation and identification as to which trace metals are limiting has been tenuous, due to the positive response to a variety of treatments, some of which contained undefined concentrations of macronutrients and trace metals, and because procedures to reduce trace metal contamination were not adhered to (Goldman 1964; Arneson 1979; Goldman 1981; Byron et al. 1983; Lane and Goldman 1984; Byron and Goldman 1988; Goldman 1988). In some instances, where known concentrations of Fe were added, chemical speciation calculations (HYDRAQL) indicated that Fe precipitates would have formed. In these cases, the possibility of a toxic substance (trace metal) adsorbing onto the newly formed precipitate cannot not be excluded. Thus the observed increases in ^{14}C uptake cannot be definitely attributed to increases in Fe availability.

The possibility that other micronutrients (e.g. Co, Mn, and Mo) play some role in limiting biomass cannot be discounted. However, considering the significant effects observed in the chelator experiments, we believed that concentrating on essential trace metals with a high affinity for organic complexation (i.e. high on the Irving-Williams order) was an appropriate strategy. For example, association constants for Co and Mn complexes with EDTA are three to five orders of magnitude lower than for Cu (Mo exists as a ligand).

Nutrient ratios also suggest that an essential nutrient other than phosphate is either colimiting or very near limiting concentrations. The typical phosphate to dry weight ratio for aquatic algae suggests that under optimum conditions, 0.03 mol of phosphate should yield 100 g dry weight of algae (Vallentyne 1974). The maximum dry weight obtained in our bioassays was 3×10^{-4} g $\cdot$ L $^{-1}$ (0.4 μM P and 0.4 μM EDTA), which, according to Vallentyne's ratio, should require only 0.094 μmol of phosphate, an order of magnitude less than what was needed. This suggests that phytoplankton may not have been able to utilize all available phosphate.

A similar calculation made for N produces a contrasting result. According to Vallentyne's ratio, if 0.5 mol of N can yield 100 g dry weight of algae, then 1.5×10^{-6} mol of N

would be needed to support 3×10^{-4} g dry weight. This is an order of magnitude higher than the amount of $\text{NO}_3\text{-N}$ available in Lake Tahoe ($\sim 2.4 \times 10^{-7}$ mol) (Fig. 6). Therefore, forms of N other than $\text{NO}_3\text{-N}$ must have been available, a conclusion supported by subsequent field measurements that indicated that approximately 75% of the total N in the water column was organic N (C. C. Y. Chang and R. Petersen, unpubl. data).

Finally, although primary productivity has reportedly become more P sensitive as total $\text{NO}_3\text{-N}$ has increased at Lake Tahoe, primary productivity decreased when total P increased by approximately 42% from 1975 to 1976 and then decreased again in 1977, although total P increased by 18% (Goldman 1988). If P concentrations truly increased, then these results can only be explained if (1) the portion that was bioavailable P did not increase or (2) an essential trace metal was limiting, and hence, an increase in P did not result in an increase in primary productivity. These results suggest that close attention must be paid to macronutrient as well as micronutrient speciation.

$\text{NO}_3\text{-N}$ Limitation

That the algae in our experiments were $\text{NO}_3\text{-N}$ limited seems unlikely. In 1989, $\text{NO}_3\text{-N}$ concentrations measured at the two stations were approximately twice as high as in 1988 ($\sim 0.42 \mu\text{M}$; Fig. 6). If the algae were N limited, yields for the controls (i.e. no solute additions) would have been higher in 1989, after lake turnover, but this was not the case (Fig. 5). Furthermore, in subsequent short-term ^{14}C bioassays done in early spring and late summer of 1991, additions of $\text{NO}_3\text{-N}$ to $\text{NH}_4\text{-N}$ to Lake Tahoe phytoplankton did not stimulate primary productivity (C. C. Y. Chang and R. Petersen, unpubl. data).

Although $\text{NO}_3\text{-N}$ may not have directly limited biomass during the course of the experiments, Fe-limiting conditions may have existed for some of the $0.4\text{-}\mu\text{M}$ EDTA treatments and all of EDDHA treatments, and led to effective N limitation, by preventing $\text{NO}_3\text{-N}$ reduction. Fe serves as a reducing agent in the reduction of $\text{NO}_3\text{-N}$ to $\text{NH}_4\text{-N}$. In addition, Fe-dependent biochemical reactions are likely affected in Fe cells (Cardenas et al. 1974; Glover 1977; Guikema and Sherman 1982; Sandmann and Malkin 1983; Storch and Dunham 1986).

Comparison between Stations

Bioassays at both stations suggested phytoplankton limitation by P and Fe, despite the obvious differences in water properties between the two stations. At the time (April and May) and depth (15 m) when or from which water was obtained for the bioassays, ATP and SPM concentrations were higher at the Index than at the Midlake station (Fig. 3; Table 3). This presumably reflects greater phytoplankton abundance at the Index station, which was not surprising, as nutrient flux to the surface from deeper water is greater at the Index station which is also closer to the mouths of streams (Abbott et al. 1984). Higher phosphate concentrations at the Index station may explain the slightly higher yields in the EDDHA bioassays for *A. italica* grown in Index station water.

Finally, as mentioned above, because Cu and Fe concentrations did not vary greatly with depth or between stations (Table 2), and because the concentrations used to estimate trace metal speciation were high relative to the rest of the water column, it is likely that trace metal activity is below toxic thresholds and that Fe-limiting conditions exist throughout the water column.

Conclusion

Because concentrations of all essential nutrients are low at Lake Tahoe, except Si ($\sim 4 \times 10^{-4}$ M), it is difficult to accurately describe the nutrient status of the community.

The results of our field sample analysis and bioassay studies suggest that phytoplankton biomass at Lake Tahoe may be phosphate limited. Secondary limitation imposed by trace metals, possibly Fe, was also indicated. Results from chemical speciation calculations using field data also suggested that Fe may have been limiting. N limitation seems less likely, although Fe-limiting conditions may impose an effective N limitation if insufficient Fe is available for $\text{NO}_3\text{-N}$ reduction. In general, this study emphasizes that in oligotrophic environments, where essential nutrients were all at low concentrations, small perturbations in water chemistry can have pronounced effects on biological characteristics.

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Complex Interactions between Fish and Zooplankton: Quantifying the Role of an Open-Water Planktivore

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An open-water planktivore, the gizzard shad (*Dorosoma cepedianum*), can drive complex interactions among fish and zooplankton in Ohio reservoirs. In Kokosing Lake, crustacean zooplankton density declined to near zero immediately after larval gizzard shad abundance peaked during 1987 and 1988. This decline can be attributed to increased death rates, due to predation, and to reduced number of eggs per cladoceran. In an enclosure/exclosure experiment, young-of-year gizzard shad at lake densities significantly reduced density of crustacean zooplankton and rotifers within 2 wk. In addition, phytoplankton that were edible to zooplankton were reduced in enclosures, likely due to a combination of direct herbivory by gizzard shad and reduced nutrient availability due to uptake by the growing gizzard shad. Gizzard shad not only directly influenced zooplankton via predation, they also indirectly affected zooplankton by reducing phytoplankton abundance. Because larval bluegill (*Lepomis macrochira*) migrated to the limnetic zone during or shortly after the zooplankton decline, food available to these zooplanktivorous larvae, as well as their ultimate recruitment, was reduced with gizzard shad. Through direct (i.e. predation) and indirect (i.e. influencing algal abundance) pathways, gizzard shad can drive zooplankton to extinction, thereby reducing recruitment of other fishes and controlling community composition.

Un planctonophage de mer libre, l'aloise à gésier (*Dorosoma cepedianum*) peut causer des interactions complexes entre les poissons et le zooplancton dans des bassins de l'Ohio. Dans le lac Kokosing, la densité du zooplancton composé de crustacés a chuté à presque zéro juste après que la population de larves d'aloses à gésier a atteint un sommet au cours des années 1987 et 1988. Cette diminution peut être attribuée à un taux de mortalité accru, en raison de la prédation et du nombre réduit d'oeufs par cladocère. Dans une expérience comparative enclos/hors enclos, de jeunes aloses à gésier de l'année en densité comparable à celle des populations lacustres, ont fait diminuer de façon importante la densité du zooplancton de crustacés et celle des rotifères en moins de deux semaines. En outre, le phytoplancton qui sert de nourriture au zooplancton a diminué dans les enclos, probablement en raison de deux phénomènes conjugués : l'aloise à gésier en a consommé directement, et elle a également ingéré des matières nutritives pendant sa croissance, réduisant ainsi la quantité de nourriture disponible pour le phytoplancton. L'aloise à gésier a nui au zooplancton non seulement de façon directe, par prédation, mais aussi indirectement, en réduisant la densité du phytoplancton. Étant donné que les larves de branchie bleue (*Lepomis macrochira*) migrent vers la zone limnétique lorsque le zooplancton se fait plus rare, ou peu après cette baisse, l'aloise à gésier a provoqué une diminution de la nourriture disponible pour ces larves zooplanctonivores, de même qu'un déclin de leur recrutement final. De façon directe (c'est-à-dire par la prédation) et indirecte (en modifiant la concentration algale), l'aloise à gésier peut provoquer la disparition du zooplancton, réduisant ainsi le recrutement d'autres poissons et limitant la composition de la communauté.

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Predators can dramatically influence aquatic communities, both directly and indirectly (see chapters in Kerfoot and Sih 1987; Carpenter 1988; Ebenman and Persson 1988). By removing prey, predators directly alter community species composition. Indirect effects of predators can take several forms, leading to positive (e.g. Kerfoot 1987) as well as negative (e.g. Mittelbach and Chesson 1987) effects for prey. The current focus within ecology on biomanipulation, applying the cascading trophic interactions hypothesis (Shapiro and Wright 1984; Carpenter et al. 1985; McQueen et al. 1989; Gophen 1990; McQueen 1990) as a tool to influence water quality, emphasizes the indirect impacts that predators can have on entire lake ecosystems, with effects of predation extending beyond adjacent trophic levels.

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Systemwide effects of predators have been well described for planktivore-plankton interactions (see reviews in Hall et al. 1976; Greene 1985; Lazzaro 1987; Northcote 1988; Gliwicz and Pijanowska 1989). In general, planktivorous fishes remove large zooplankters (e.g. Brooks and Dodson 1965), and invertebrates remove small forms (e.g. Wong 1981; Dodson 1974). The conventional model states that when planktivorous fish occur in a lake, large zooplankters are selectively removed and smaller forms dominate (e.g. Brooks and Dodson 1965; Wells 1970; Warshaw 1972; Evans 1986). Furthermore, because vertebrate predators select large prey, they also will remove invertebrate predators, which tend to be large, reducing their predation on small zooplankton and reinforcing the dominance of small forms (Dodson 1970, 1974). Without fish, competitive interactions among plankters are complex, influenced by body size and environmental conditions (Bengtsson 1987; DeMott

1989); however, because invertebrate predators can survive and remove small zooplankters (Lynch 1979), large forms tend to predominate.

Studies detailing these interactions typically have involved adult fishes; however, larvae of most fishes also feed on zooplankton (e.g. Applegate and Mullan 1967; Werner 1969; Keast 1980; Whiteside et al. 1985a, 1985b; Whiteside 1989) and can be important regulators of zooplankton abundance (Doolittle 1982; Whiteside et al. 1985b; Cryer et al. 1986; Mills et al. 1987; Whiteside 1989). Unlike adults, larval planktivores are gape-limited predators, initially removing small plankters and including larger prey as they grow and their gape increases (Rosenthal and Hempel 1970; Wong and Ward 1972; Zaret 1980; Hansen and Wahl 1981). Seasonality in fish spawning produces pulses of larval planktivores resulting in seasonal impacts on zooplankton. In addition, fishes may exhibit ontogenetic niche shifts to or from planktivory (Mittelbach 1981; Werner et al. 1983; Werner and Gilliam 1984), providing additional temporal variability in predation pressure on zooplankton. In turn, because year-class strength in most fishes is set during early life history (Cushing 1975; Lasker 1975; Ware 1980; Mills and Forney 1988), survival through the larval stage is critical to recruitment. Consequently, understanding the larval fish – zooplankton interaction contributes to understanding the mechanisms underlying adult population size (as well as the role of larval fishes in biomanipulation) and provides insight into community composition in lakes.

As planktivores, gizzard shad (*Dorosoma cepedianum*), which dominate the planktivore biomass of many central and southern U.S. lakes and reservoirs (Jenkins 1967; Timmons et al. 1978; Johnson et al. 1988), can influence zooplankton dynamics. Using experiments in ponds and laboratory pools, Drenner and his colleagues have documented that adult gizzard shad reduce densities of most easily captured zooplankters (Drenner et al. 1978, 1982a, 1982b, 1986; Drenner and McComas 1980; Threlkeld and Drenner 1987) and influence phytoplankton community structure (Drenner et al. 1984, 1986). All studies to date, however, have dealt with fish >50 mm (total length), providing no information on effects of particulate-feeding larvae.

Herein, we quantify how young-of-year gizzard shad (<50 mm) influence reservoir community structure, as mediated through their influence on zooplankton. We first document zooplankton and larval gizzard shad abundance patterns in an Ohio reservoir, suggesting two hypotheses to explain these patterns. Then, we describe an enclosure/exclosure experiment designed to test the validity of these hypotheses. Our results suggest that young-of-year gizzard shad can be important regulators of zooplankton, via a combination of the direct effects of predation and indirect effects on phytoplankton. Finally, we explore how gizzard shad – zooplankton interactions influence other species with zooplanktivorous larvae.

Methods

Study Lake

Kokosing Lake (Knox County, central Ohio) is a 65-ha shallow (mean depth = 2.0 m, maximum depth = 4.9 m), flood control impoundment with 7.5 km of shoreline and Secchi depths typically <1 m. Neither submersed nor emergent vegetation is abundant. The fish community consists of gizzard shad, largemouth bass (*Micropterus salmoides*), white crappie (*Pomoxis annularis*), bluegill (*Lepomis macrochira*; Bailey and Robins 1988), and common carp (*Cyprinus carpio*).

Field Sampling Methods

Larval fish were collected in two replicate daytime surface tows with a 0.75-m-diameter ichthyoplankton net (2 m long, 500- μ m mesh) towed in the limnetic zone at ≥ 1.5 m/s once per week during April through September 1986–89. We used a flowmeter mounted in the mouth of the net to calculate volume of water filtered. Larvae were preserved in formalin and returned to the laboratory where they were identified, measured (nearest millimetre, up to 50 per species), and their diets quantified (1–10 individuals per species per date, minimum number determined by availability of larvae in samples). Cladocerans and rotifers were identified to genus, and copepods were classified as nauplii, calanoids, or cyclopoids. All prey were measured (nearest 0.1 mm) and lengths were converted to biomass using taxon-specific length – dry weight regressions (G. G. Mittelbach, unpubl. data). Integrated zooplankton samples (four columns per sample, three replicate samples per date) were collected each week during April through September 1987 and 1988 using a 2-m tube sampler (7.30-cm inside diameter, 54- μ m mesh; DeVries and Stein 1991) simultaneous with larval fish collection. Samples were preserved in 5% sucrose formalin (Haney and Hall 1973) and returned to the laboratory. When fewer than 200 individuals per taxon were captured, all were counted in the sample; 10% subsamples were counted for abundant taxa until at least 200 individuals were counted. At least 20 individuals of each taxon in a sample were measured (nearest 0.1 mm) using a drawing tube and digitizing tablet. Cladocerans were measured from the anterior portion of the carapace to the base of the basal spine; copepods were measured from the anterior portion of the carapace to the base of the caudal rami. Number of individuals carrying eggs out of 50 randomly chosen individuals and the clutch size for 20 individuals carrying eggs were recorded for *Bosmina* and *Daphnia* (the dominant cladocerans) for four to six dates during and after the crustacean zooplankton decline.

To evaluate the potential for competition between larval bluegill and larval gizzard shad that cooccurred in the limnetic zone (larval bluegill spend several weeks in the limnetic before returning to the littoral zone, Werner 1967; Storck et al. 1978), we used Schoener's overlap index (Schoener 1970) based on the average proportion that each prey taxon contributed to total biomass in larval fish diets (i.e. the proportion was calculated within each fish and averaged across fish within a date, Wallace 1981). The formula for this index is

$$\text{Overlap} = 1 - 0.5 \left(\sum_{i=1}^n |r_{xi} - r_{yi}| \right)$$

where r_{xi} and r_{yi} = the proportions of prey type i in the diets of species x and y , respectively, and n = number of food categories. This index ranges from 0 to 1, with 0 representing no diet overlap and 1 indicating complete diet overlap. Because larval fish and zooplankton communities changed through time, we treated overlap measures calculated for each sample date as replicates.

To evaluate prey selection by fishes, we compared larval fish diets with zooplankton samples using Chesson's alpha (Chesson 1978, 1983), treating individual fish as replicates within each date. The formula for this index is

$$\alpha = \frac{r_i/p_i}{\sum_i (r_i/p_i)}, \quad i = 1, \dots, m$$

where p_i = the proportion of prey item i in the environment (i.e. the lake), r_i = the proportion of prey item i in the fish's diet, m = the number of prey items in the environment, and α = the selectivity. With this index, a value of $\alpha = (1/m)$ occurs when a prey item is consumed in proportion to its abundance in the environment; values $> 1/m$ indicate preference and values $< 1/m$ indicate avoidance.

Enclosure/Exclosure Experiment

To quantify the impact of larval gizzard shad on zooplankton community structure, we conducted an enclosure/exclosure experiment in Kokosing Lake during 6 June through 5 July 1988. Twelve polyethylene bags (1.13-m diameter, 2 m deep, 2-m³ volume; not in contact with the sediment, bags were sealed at the bottom) were filled with lake water from a depth of 1 m using a large water pump (3.8-cm hose diameter, 189 L/min) on 16–17 May 1988 (just before larval gizzard shad were first collected in the limnetic zone). On 6 June 1988, larval gizzard shad were collected at night by attracting them to lights (Gregory and Powles 1985) and stocked into six randomly chosen bags at a density of 120 fish/bag. Mean size of stocked fish was 18.4 ± 0.4 mm total length (TL) (mean ± 1 SE, $n = 38$). To estimate mortality due to handling of stocked larval gizzard shad, we introduced 50 fish into each of two containers (about 200 L) floating next to the bags. The next day, these containers were drained; surviving fish were counted and measured. Survival of stocked gizzard shad larvae, based on survival in our holding containers, was 32% (range 29–35%, $n = 2$), yielding a stocking density of 19.4 fish/m³ (mean biomass ± 1 SE = 7.15 ± 0.01 kg/ha), similar to the peak density (13.9 fish/m³) in Kokosing Lake during 1988, but much lower than the 1987 peak (67.3 fish/m³, see Results). To control for nutrient release in enclosures due to larval shad mortality (Threlkeld 1987), 100 dead larval gizzard shad (i.e. the number we expected a priori to die in enclosures due to handling stress, based on preliminary experiments) were added to each exclosure. On 7 June 1988 and weekly thereafter, zooplankton were sampled from the bags with a 2-m integrated tube sampler. Procedures for zooplankton sampling and processing were as for lake samples. Phytoplankton samples were collected from three randomly chosen enclosures and exclosures on 7 and 21 June 1988 (i.e. the beginning and midpoint of the experiment) by taking 100 mL of water from an integrated sample (collected using the 2-m tube sampler, but without filtering through the 54- μ m net) and preserving with Lugol's preservative. These 100-mL samples were concentrated (20 $\times$) by settling for 24–48 h and two transects across a Sedgewick–Rafter cell were counted for each sample. On 5 July 1988, bags were drained using the large water pump; all fish were collected and preserved. All data were analyzed using ANOVA and repeated-measure ANOVA techniques (GLM procedure, SAS Institute Inc. 1985).

Results

Field Pattern: Larval Gizzard Shad and Zooplankton

Larval gizzard shad abundance peaked during late May to early June (Fig. 1A, 1E, and 7A–7D). Larvae were first captured during mid- to late May, with peak densities occurring 1–2 wk after first capture. Peak density varied between years, ranging from 14 fish/m³ during 1988 to 84 fish/m³ during 1986 (see Fig. A1, 1E, and 7A–7D).

Although crustacean zooplankton abundance differed between 1987 and 1988 (repeated-measures ANOVA, $F_{1,4} =$

64.49, $p = 0.001$), the overall pattern of a single peak during spring, followed by a dramatic decline, was similar in both years (Fig. 1B and 1F; the time $\times$ year interaction term was significant, $F_{20,80} = 15.58$, $p = 0.0002$, likely due to increasing abundance during October 1987). No single taxon dominated the crustacean zooplankton during either year, although copepod nauplii were numerically important during 1988 (Fig. 1C, and 1G). Rotifer abundance patterns, as well as absolute densities, varied between years (year effect, $F_{1,4} = 15.69$, $p = 0.02$; time $\times$ year interaction, $F_{20,80} = 18.92$, $p = 0.0002$). However, rotifer density did not consistently decline each year as did the crustacean zooplankton (Fig. 1D, and 1H). In both 1987 and 1988, the period of most rapid zooplankton decline occurred within 2 wk of peak larval gizzard shad density.

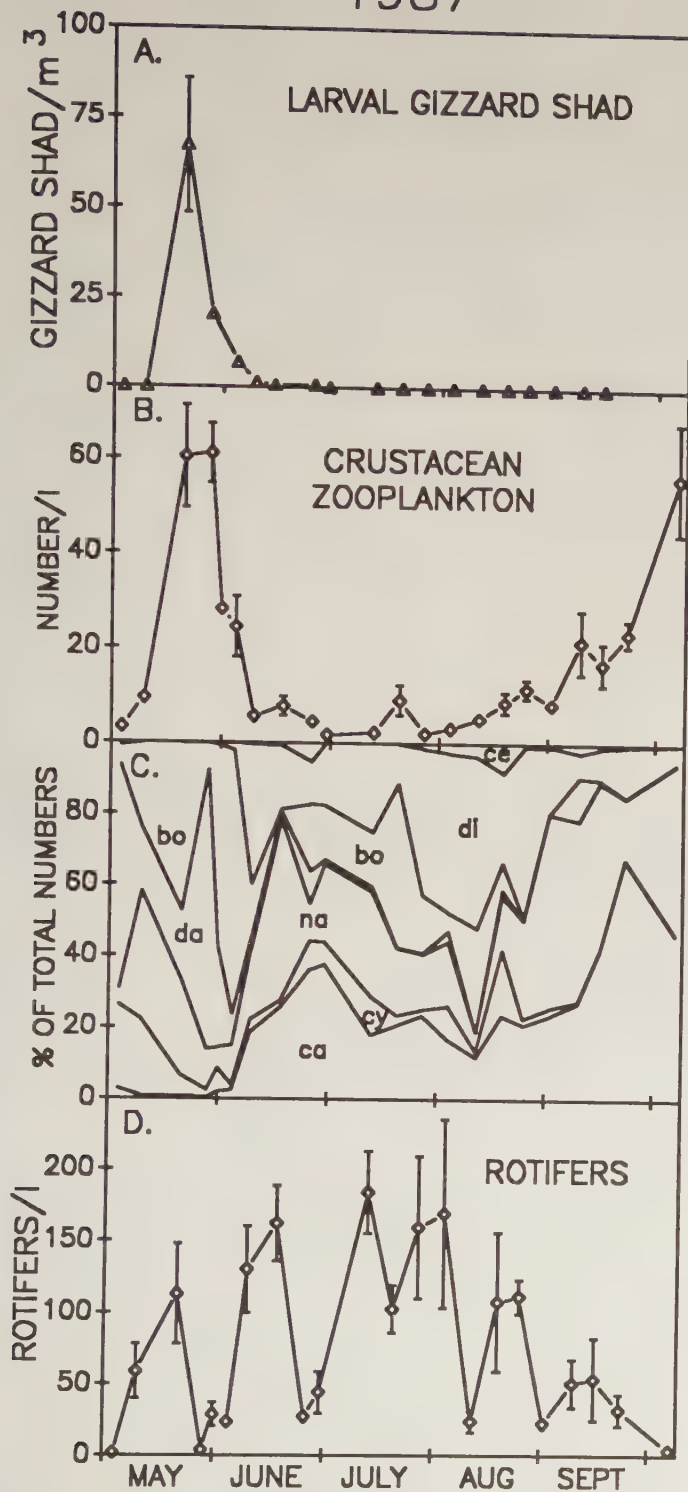
Declining zooplankton abundance could be caused by decreased zooplankton reproduction as well as by increased predation due to young-of-year gizzard shad. To evaluate whether zooplankton reproduction was reduced during the period of declining density, we regressed an index of fecundity for *Bosmina* and *Daphnia*, the dominant cladocerans, on time during and just after the decline in zooplankton density in both years (22 May to 10 June 1987, 24 May to 27 June 1988; Fig. 1B, and 1F). The index was calculated as the product of the proportion of individuals that carried eggs and the mean number of eggs per individual carrying eggs. We included dates from during and just after the zooplankton decline, testing the null hypothesis that zooplankton reproduction declined during this period, contributing to the decline. The fecundity index for both taxa did not change during 1987 (Fig. 2A; linear regression, $p > 0.08$ for both *Bosmina* and *Daphnia*). During 1988 the fecundity index declined for both taxa during the crustacean zooplankton crash (Fig. 2B; linear regression $p < 0.01$ for both taxa).

Enclosure/Exclosure Experiment

Fish survival (exclusive of preexperiment handling mortality) varied across enclosures (ranging from 32 to 82%), yielding densities at experiment's end of 6–15.5 fish/m³ (mean ± 1 SE = 10.9 ± 1.4 fish/m³). Thus, a total of 89–108 (from initial stocking to end) fish died in enclosures, similar to the 100 dead larval gizzard shad that were added to exclosures. In addition, two exclosures contained 1.5 and 5.5 fish/m³ at experiment's end. Because these bags were covered during the experiment and had no holes at draining, fish must have been introduced as exclosures were filled. Because fish were predicted to reduce zooplankton density, their presence in "exclosures" generates a conservative test; thus, data from these exclosures were included in all analyses as fish-exclosure data. At experiment's end, fish averaged 40.7 ± 2.1 mm TL (mean ± 1 SE), and final length was negatively related to final gizzard shad density (Fig. 3; linear regression, $F_{1,6} = 16.88$, $p = 0.006$). This relationship was significant regardless of whether data from the two exclosures with fish were included. Fish biomass in enclosures was 70.3 ± 7.0 kg/ha (mean ± 1 SE, range = 40.3–94.2 kg/ha) at experiment's end.

Crustacean zooplankton density declined in enclosures relative to exclosures (Fig. 4A; repeated-measures ANOVA, treatment effect, $F_{1,10} = 76.00$, $p = 0.0001$; time $\times$ treatment interaction, $F_{4,40} = 35.74$, $p = 0.0001$); in fact, differences existed after only 2 wk, when zooplankton density in enclosures was reduced by 95% (ANOVA, $F_{1,10} = 39.55$, $p = 0.0001$). Although initial crustacean zooplankton density was marginally higher in bags with fish than in the lake (ANOVA,

1987



1988

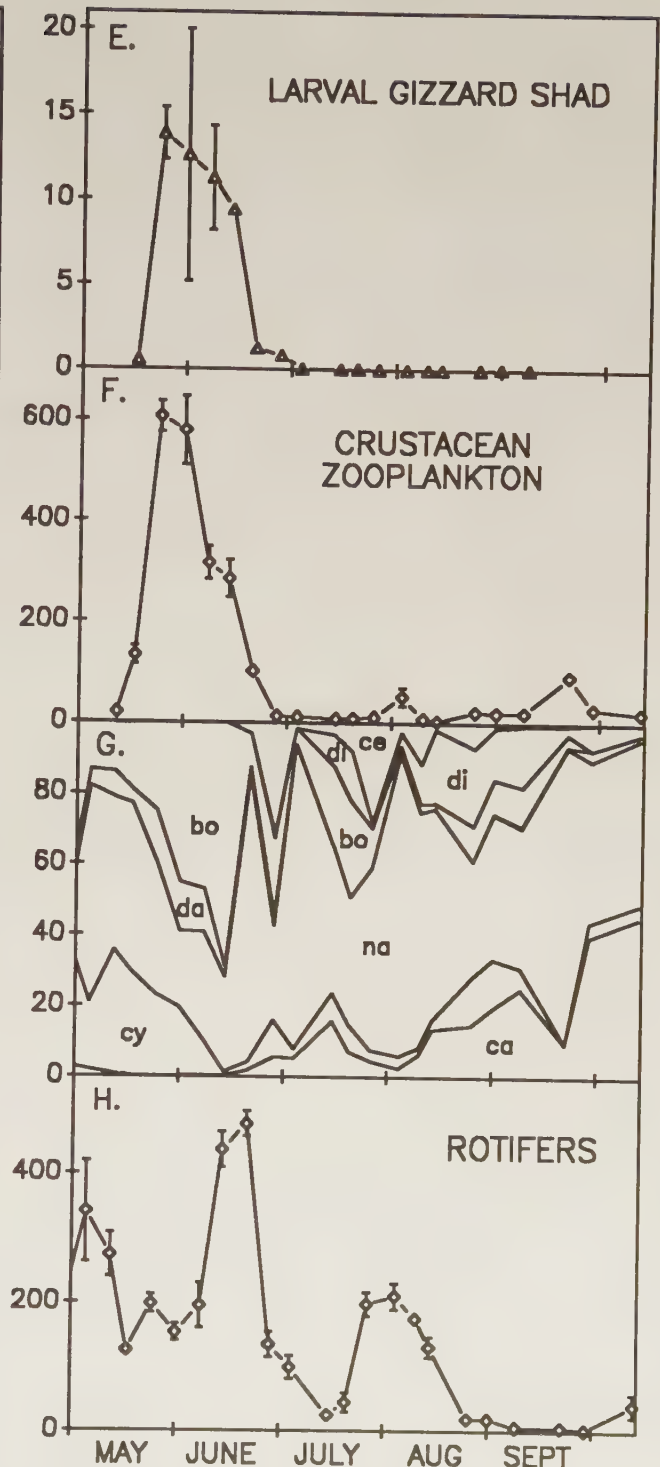


FIG. 1. Abundance of (A and E) larval gizzard shad and (B and F) crustacean zooplankton, (C and G) crustacean zooplankton species composition, and (D and H) abundance of rotifers in Kokosing Lake, Ohio, during 1987 and 1988. Larval fish data are means $\pm$ 1 SE from two replicate surface tows each week; zooplankton and rotifer data are means $\pm$ 1 SE from three replicate integrated tube samples each week. Note different y-axis scales among panels. Abbreviations for zooplankton taxa: ca = calanoid copepods, cy = cyclopoid copepods, na = copepod nauplii, da = *Daphnia*, bo = *Bosmina*, di = *Diaphanosoma*, and ce = *Ceriodaphnia*.

$F_{1,7} = 7.99$, $p = 0.03$, p required for significance = $(0.05/5) = 0.01$, density declined similarly in the lake and enclosures (Fig. 4A; repeated-measures ANOVA, treatment effects $F_{1,7} = 1.71$, $p = 0.23$). Higher initial density in bags versus the lake occurred because bags excluded all planktivores for the

3 wk between filling and fish addition. Rotifer densities also declined in enclosures relative to enclosures (Fig. 4C; repeated-measures ANOVA, treatment effect, $F_{1,10} = 26.76$, $p = 0.0004$; treatment $\times$ time interaction, $F_{4,40} = 19.58$, $p = 0.0001$), differing between treatments 2 wk after fish introduc-

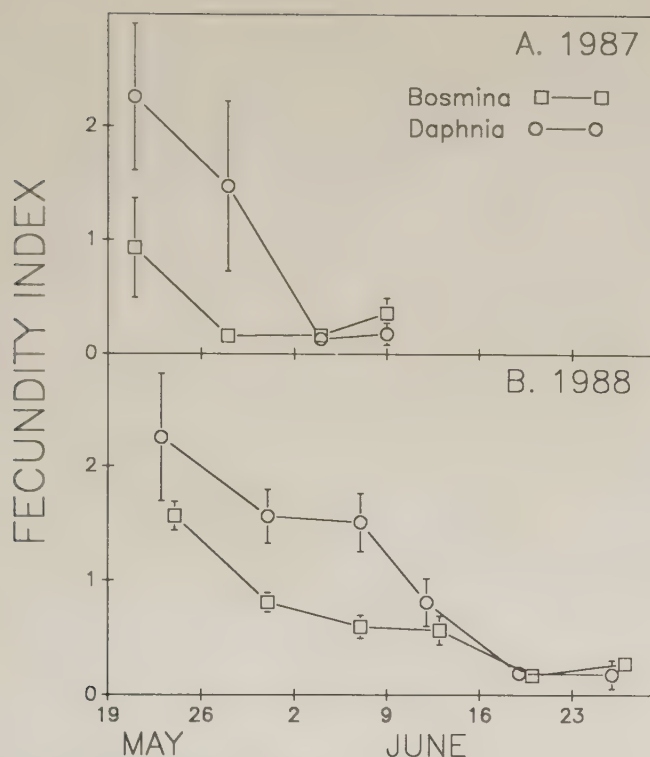


FIG. 2. Fecundity index for *Bosmina* (squares) and *Daphnia* (circles) in Kokosing Lake during the (A) 1987 and (B) 1988 zooplankton peaks and declines (22 May to 10 June 1987 and 24 May to 27 June 1988; see Fig. 1B and 1F). The fecundity index was calculated as the product of the proportion of individuals carrying eggs ($n = 50$) and the mean number of eggs per individual carrying eggs ($n = 20$). Data are means ± 1 SE from three replicate samples.

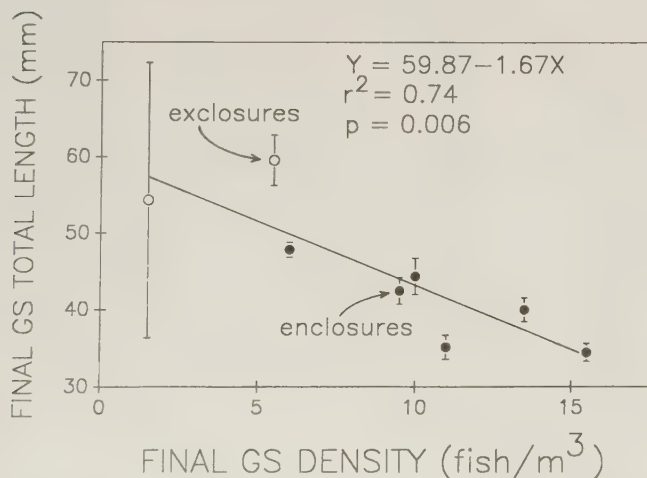


FIG. 3. Final size (± 1 SE) of gizzard shad (GS) from the 1988 bag experiment in Kokosing Lake. Solid circles represent data from enclosures ($n = 6$) and open circles are from "exclosures" ($n = 2$ of 6 exclosures) that had fish at the end of the experiment.

tion (Fig. 4C; ANOVA, $F_{1,10} = 6.75$, $p = 0.027$), a difference that became larger through time.

Crustacean zooplankton size differed between treatments (Fig. 4B; repeated-measures ANOVA, treatment effect, $F_{1,10} = 7.88$, $p = 0.02$; treatment $\times$ time interaction, $F_{4,40} = 6.82$, $p = 0.002$). Size did not differ between treatments at the beginning or end of the experiment (ANOVAs, $p > 0.11$), but zooplankton were smaller in enclosures 2 and 3 wk after the fish addition (ANOVAs, $p < 0.003$). However, zooplankton size

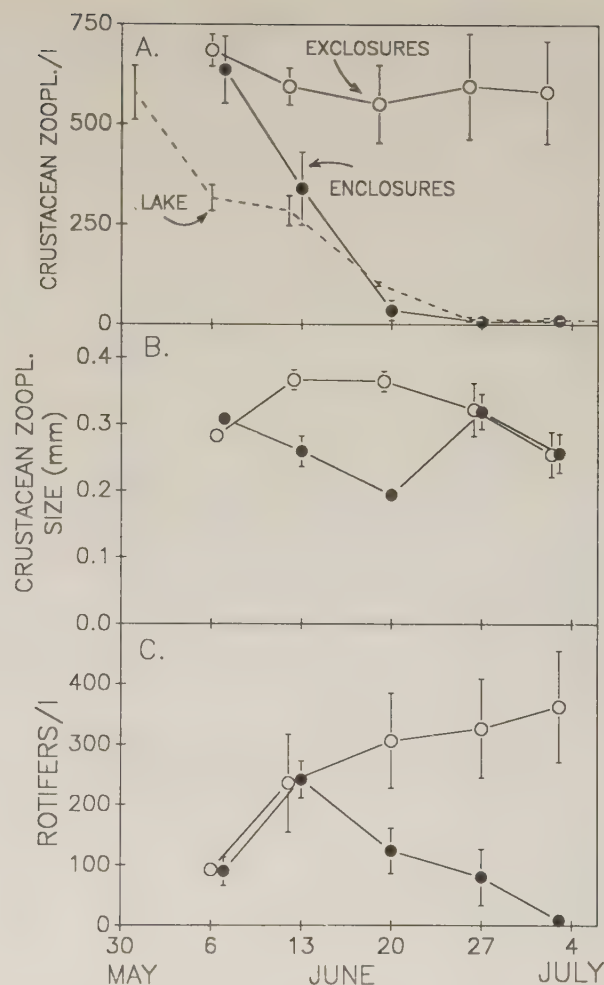


FIG. 4. (A) Density of crustacean zooplankton, (B) size of crustacean zooplankton, and (C) density of rotifers in enclosures (solid circles) and exclosures (open circles) from the 1988 bag experiment in Kokosing Lake. Included in Fig. 4A is crustacean zooplankton density from Kokosing Lake (broken line). Data are means ± 1 SE from six replicates in bags and three replicates in the lake.

within taxa did not differ (repeated-measures ANOVA, treatment effects, all $p > 0.07$ for *Bosmina*, *Ceriodaphnia*, *Daphnia*, calanoid copepods, cyclopoid copepods, and copepod nauplii).

As discussed earlier, declining zooplankton abundance could result from reduced zooplankton birth rates as well as from increased predation pressure by gizzard shad. For *Bosmina*, the only date on which mean fecundity differed between treatments was on 27 June 1988, when fecundity was higher in exclosures (Fig. 5A). Mean fecundity of *Daphnia* declined similarly in both treatments (repeated-measures ANOVA, time $\times$ treatment interaction, $F_{4,40} = 0.85$, $p > 0.48$) but was lower in enclosures than in exclosures (treatment effect, $F_{1,10} = 6.07$, $p = 0.03$) (Fig. 5B).

Volume of phytoplankton that was edible to zooplankton in the bags, primarily *Cryptomonas*, *Cosmarium*, *Chroomonas*, and very small cells, was reduced in enclosures relative to exclosures (Fig. 6; repeated-measures ANOVA, treatment effect, $F_{1,4} = 12.55$, $p = 0.02$; time $\times$ treatment interaction, $F_{1,4} = 12.67$, $p = 0.02$). However gizzard shad did not influence volume of inedible phytoplankton (primarily *Oocystis*, *Pediastrum*, *Synedra*, and *Fragellaria*; $p > 0.17$ for both the treatment effect and the time $\times$ treatment interaction). Because

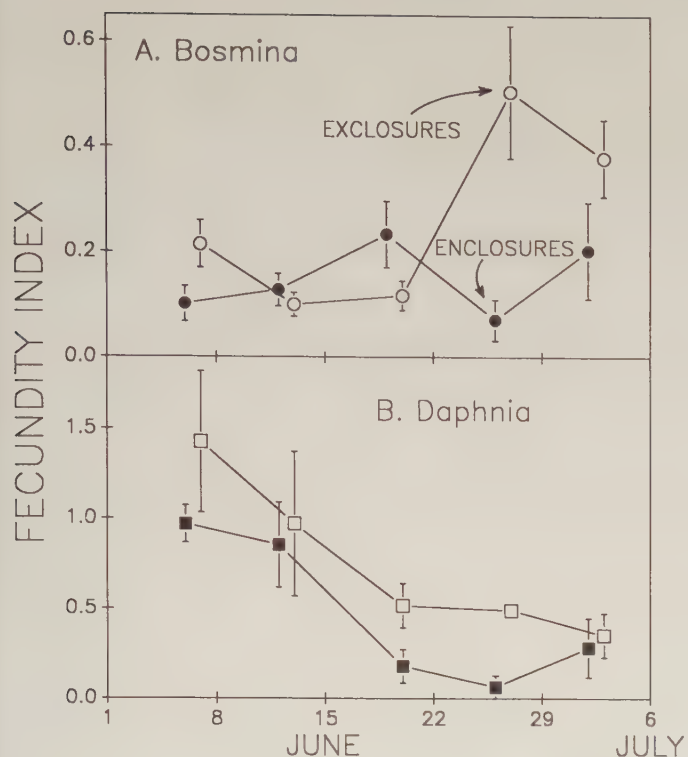


FIG. 5. Fecundity index for (A) *Bosmina* and (B) *Daphnia* in enclosures (solid symbols) and exclosures (open symbols) during the 1988 experiment in Kokosing Lake. The fecundity index is calculated as the product of the proportion of individuals carrying eggs and the mean number of eggs per individual carrying eggs. Data are means ± 1 SE from six replicates.

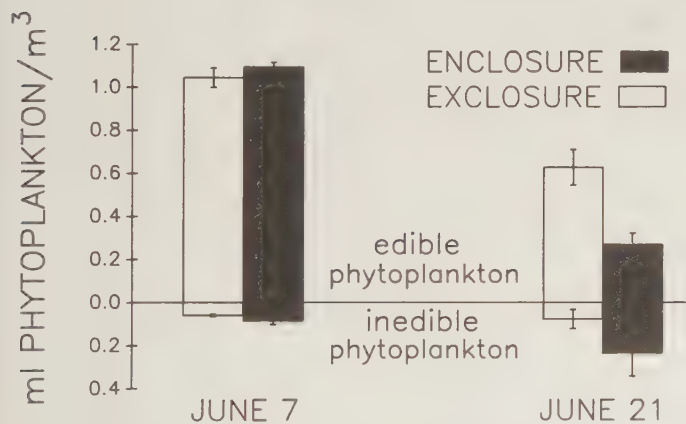


FIG. 6. Biovolume of edible and inedible phytoplankton in enclosures (solid bars) and exclosures (open bars) at the beginning (7 June 1988) and middle (21 June 1988) of the bag experiment in Kokosing Lake. Data are means ± 1 SE from three replicates.

75–90% of edible phytoplankton, at least on 7 June and in exclosures on 21 June, was *Cryptomonas*, the response of edible phytoplankton was due to a reduction in *Cryptomonas* in enclosures relative to exclosures. Using equations in Reynolds (1984) and assuming that carbon content equals 50% phytoplankton dry weight (Reynolds 1984), carbon content of edible phytoplankton in the bags was 0.200 ± 0.008 and 0.209 ± 0.04 mg/L (mean ± 1 SE) in exclosures and enclosures, respectively, at the start of the experiment. On 21 June, 2 wk into the experiment, carbon content was 0.121 ± 0.016 mg/L in exclosures and 0.053 ± 0.010 mg/L in enclosures.

Field Patterns: Larval Bluegill

Bluegill spawned for up to 3 mo of each year during 1986–89, as documented by presence of limnetic larvae. Larval bluegill and gizzard shad typically overlapped in the limnetic zone for only 2–4 wk during each year of 1986–89 (Fig. 7). However, because bluegill spawned later than gizzard shad, larval bluegill initially arrived in the limnetic zone as crustacean zooplankton were declining precipitously (cf. Fig. 1B and 1F and 7B and 7C).

Small larval bluegill (i.e. ≤ 6.9 mm) preferred small prey (nauplii and rotifers) during both years, while slightly larger fish (7.0–9.9 mm) showed no significant preference during 1987 and selected *Bosmina* during 1988 (Fig. 8). Bluegill ≥ 10 mm did not feed preferentially on any prey taxa during 1987, but selected *Diaphanosoma* during 1988 (Fig. 8). Variability in alphas was high, in part, because sample sizes were small, given that few bluegill were collected in offshore larval tows, particularly during 1987.

Larval gizzard shad < 10 mm preferred copepod nauplii in both years, and fish 7.0–9.9 mm preferred cyclopoid copepods during 1988 (Fig. 8). Gizzard shad 10.0–12.9 mm showed no preference during 1987 and selected copepod nauplii and cyclopoid copepods during 1988. Fish ≥ 13.0 mm selected cyclopoid copepod and rotifers during both years (Fig. 8). In addition, phytoplankton was observed in larval gizzard shad diets, although poor preservation techniques for fish prohibited us from quantifying or identifying algae. Phytoplankton was never observed in larval bluegill diets. Overlap between larval bluegill and gizzard shad, measured using Schoener's index, and based only on zooplankton in fish diets (i.e. excluding phytoplankton), was 0.52 ± 0.16 (mean ± 1 SE, $n = 4$ dates) during 1987 and 0.52 ± 0.05 (mean ± 1 SE, $n = 3$ dates) during 1988.

To evaluate how larval fish responded to the decline of crustacean zooplankton, we quantified diets of 15-mm gizzard shad (size held constant to control for changes in diet due to fish size) before, during, and after the decline during 1987 and 1988. Because biomass of prey consumed by larval gizzard shad does not change during daylight hours (Dettmers and Stein 1992), biomass of prey in larval shad stomachs provides a relative estimate of consumption, based on a single collection. Diets were quantified from five dates during both years: two during the crustacean zooplankton peak, two during the decline, and one after the decline. Biomass of prey in larval gizzard shad stomachs differed across sample dates ($F_{4,40} = 5.97$, $p = 0.0007$) and differed marginally between years (Fig. 9A; two-way ANOVA, year effect, $F_{1,40} = 3.39$, $p = 0.07$; year $\times$ date interaction, $F_{4,40} = 4.23$, $p = 0.006$). During 1987, prey biomass was greatest during peak crustacean zooplankton abundance and decreased as crustacean zooplankton density declined (ANOVA, $F_{4,20} = 5.27$, $p = 0.005$); however, during 1988, prey biomass did not differ across sample dates (ANOVA, $F_{4,20} = 1.92$, $p = 0.15$). Peak prey biomass differed marginally between years ($F_{1,8} = 4.46$, $p = 0.07$). Further, the proportion of crustacean zooplankton in gizzard shad diets (the remainder being rotifers) decreased during and after the zooplankton crash in both years (Fig. 9B).

Based on these results, we predicted that the high peak densities of gizzard shad would lead to reduced crustacean zooplankton density, which, in turn, would reduce available food, growth, and, ultimately, recruitment of larval bluegill. Using data from 4 yr of sampling (1986–89), total larval bluegill catch-per-effort (total number of bluegill collected divided by

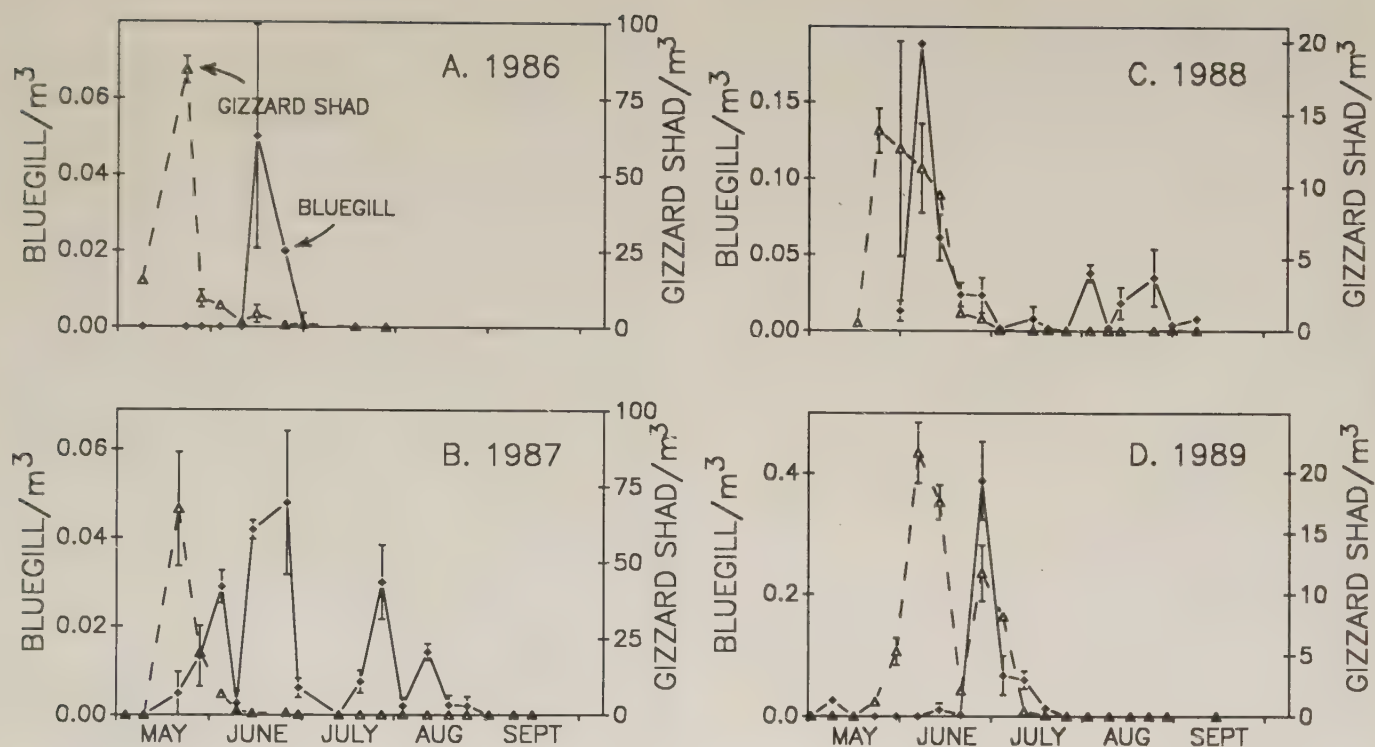


FIG. 7. Density of larval bluegill (solid diamonds, solid lines) and larval gizzard shad (open triangles, broken lines) for 1986–89. Note scale differences for the y-axes among panels.

total sampling time) was marginally related to peak larval gizzard shad density (Fig. 10; $F_{1,2} = 13.72$, $p = 0.066$).

Discussion

Larval Gizzard Shad – Zooplankton Interactions

Complex interactions among larval gizzard shad, zooplankton, and larval bluegill drive reservoir community dynamics during spring in Kokosing Lake. Via an enclosure/exclosure experiment, we demonstrated that gizzard shad dramatically reduce crustacean zooplankton and then switch to rotifers shortly thereafter, in enclosures. In exclosures, densities of all zooplankton remained high. Patterns in the lake followed those in enclosures, suggesting that we mimicked lake conditions in this treatment.

At the start of the enclosure/exclosure experiment, fish in enclosures were of a size to be feeding as particulate planktivores (Drenner et al. 1982a; Lazzaro 1987). Consistent with the prediction that fish using this feeding mode should select large zooplankton, crustacean zooplankton were smaller in enclosures than in exclosures 1–2 wk after fish introduction. By the midpoint of the experiment, fish should have grown through a size at which they should begin filter feeding (i.e. 25 mm; Drenner et al. 1982a). As filter feeders, gizzard shad consume large phytoplankton (i.e. *Ceratium*) as well as zooplankton that are most easily captured and will enhance small (<70 μm) phytoplankton (Drenner et al. 1978, 1982a, 1982b, 1984, 1986; Drenner and McComas 1980; Threlkeld and Drenner 1987). Because crustacean zooplankton densities were nearly zero 2 wk after fish introduction, we cannot evaluate whether more easily captured taxa were reduced at this point (i.e. when fish should have been >25 mm). However, phytoplankton in our experiment were depressed, rather than enhanced, in the presence of gizzard shad. Two factors are likely responsible for this reduced phytoplankton abundance

with young-of-year gizzard shad. First, biomass of gizzard shad increased by an order of magnitude in enclosures; these growing fish may have incorporated sufficient nutrients into their growing bodies to limit growth of phytoplankton (Kitchell et al. 1975; C. Kraft, University of Wisconsin, Sea Grant Institute, pers. comm.). Such increases in biomass are well within the range of those observed from a variety of systems (average gizzard shad biomass = 101 kg/ha, Jenkins 1967; maximum biomass = 1236 kg/ha, Schoonover and Thompson 1954), suggesting that nutrient limitation may operate in the field. In addition, given that young-of-year gizzard shad in Kokosing Lake consumed phytoplankton, direct herbivory by young-of-year gizzard shad likely contributed to the phytoplankton decline (see also Iwata 1977; Juario and Storch 1984).

The zooplankton decline was associated with both increased predation on zooplankton (due to increased larval fish abundance) and a decline in the fecundity index (during one of the years). Upon becoming sufficiently abundant, zooplankton overgraze phytoplankton with a commensurate decline in fecundity (Lampert et al. 1986; reviewed in Sommer et al. 1986). However, this overgrazing seems unlikely in the enclosure/exclosure experiment. In fact, fecundities in exclosures with high zooplankton densities exceeded those in enclosures with low zooplankton densities. Predation, via selective removal of the more visible, egg-bearing individuals by larval gizzard shad, can reduce apparent birth rates (Gliwicz 1981). However, because zooplankton size within any taxon did not differ between treatments, gizzard shad did not shift zooplankton population structure toward nonreproductive (i.e. smaller) individuals. Alternatively, falling fecundity in the bags could be the result of reduced phytoplankton, driven to low levels by gizzard shad (via both herbivory and nutrient limitation; see above). Because phytoplankton carbon content in enclosures was reduced to below threshold levels required for egg production by most crustacean zooplankton (e.g.

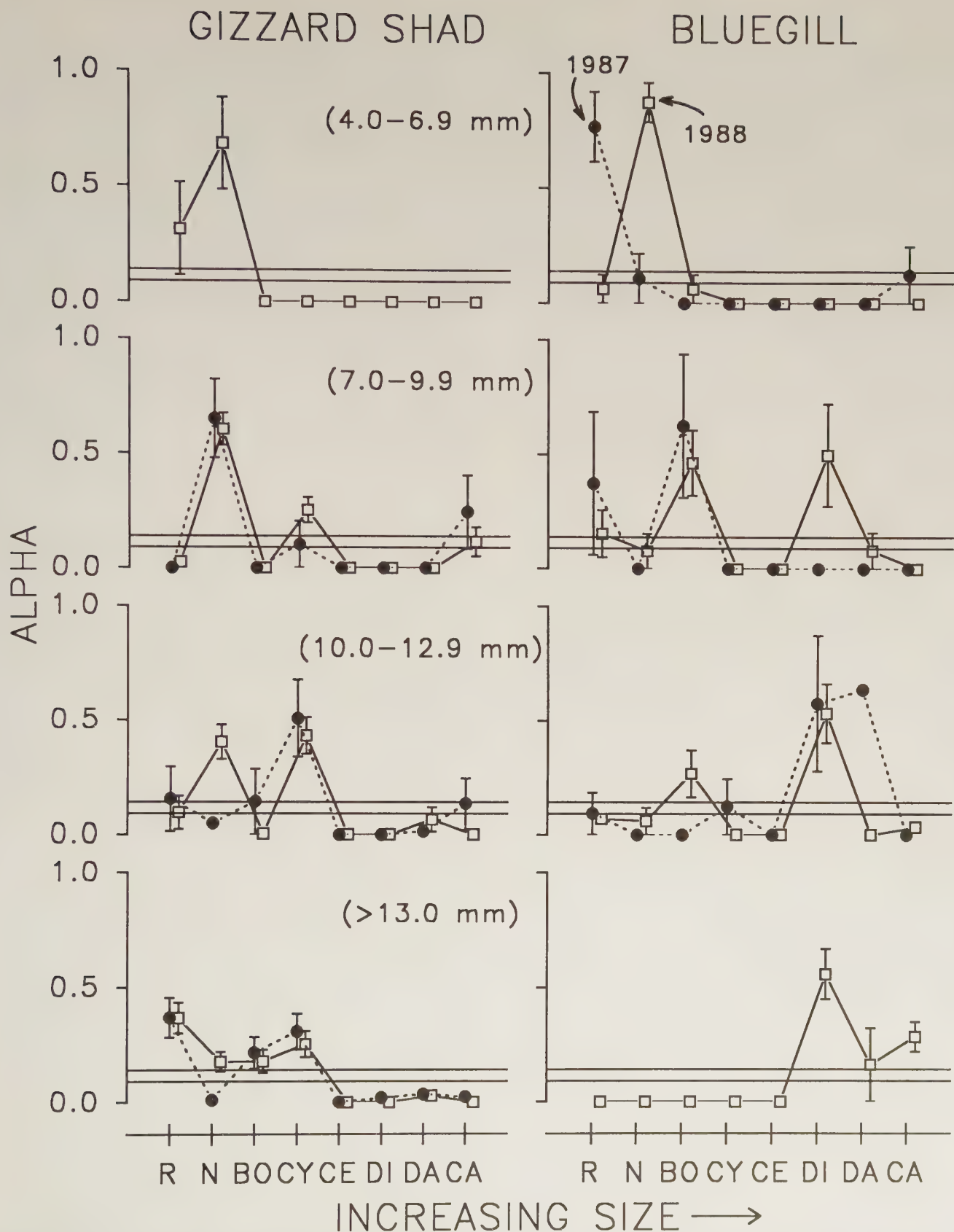


FIG. 8. Food selection (using Chesson's alpha, Chesson 1978, 1983) by larval gizzard shad and larval bluegill collected offshore in Kokosing Lake during 1987 (solid circles, broken lines) and 1988 (open squares, solid lines). Panels represent selection by different sizes of fish (4.0–6.9, 7.0–9.9, 10.0–12.9, and ≥ 13.0 mm). Data are presented as means ± 1 SE. Prey taxa are ordered along the x-axis in increasing size from left to right. Abbreviations for zooplankton taxa: R = rotifers, N = copepod nauplii, BO = *Bosmina*, CY = cyclopoid copepods, CE = *Ceriodaphnia*, DI = *Diaphanosoma*, DA = *Daphnia*, and CA = calanoid copepods. The horizontal lines indicate the region between 0.09 and 0.14, the reciprocal of the range (7–11) bracketing the number of prey taxa in the lake; values above this range indicate positive selection, and values below this range indicate avoidance. Gizzard shad 4.0–6.9 mm did not contain food during 1987, and bluegill ≥ 13.0 mm were not collected during 1987.

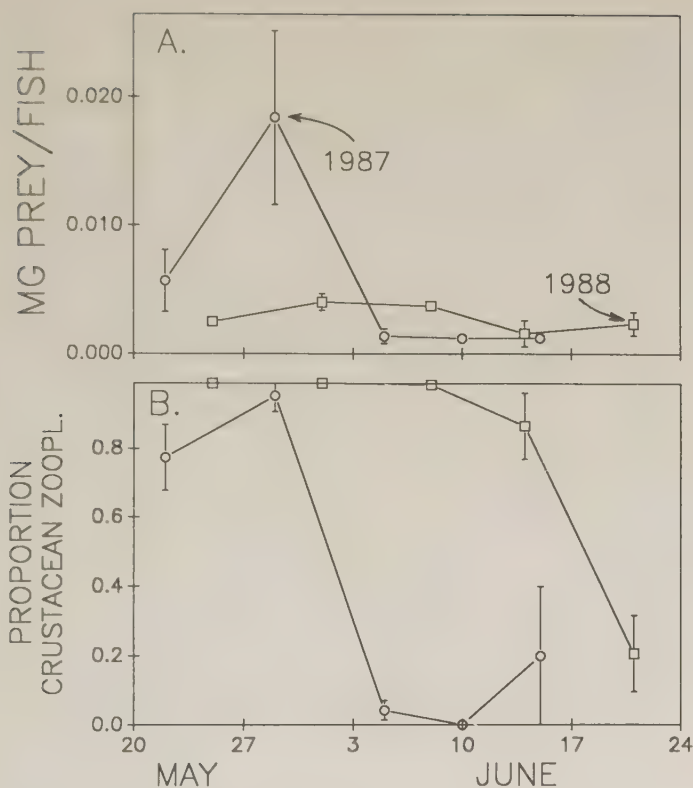


FIG. 9. (A) Mean prey biomass (dry weight) and (B) proportion (by numbers) of crustacean zooplankton in the diets of 15-mm gizzard shad ($n = 5$ fish/date) before, during, and after the 1987 (circles) and 1988 (squares) zooplankton declines in Kokosing Lake.

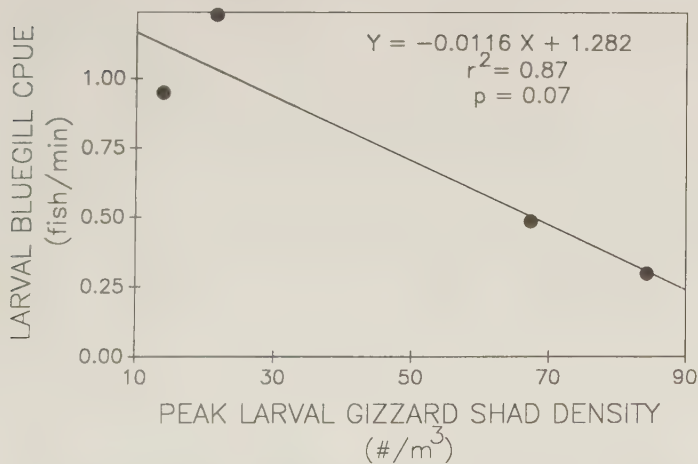


FIG. 10. Regression of number of larval bluegill captured per minute of larval fish tow (total number of bluegill caught per total sampling time) on peak larval gizzard shad density during 1986–89 in Kokosing Lake.

Lampert 1978; Gliwicz and Lampert 1990) and rotifers (Stemberger and Gilbert 1985), larval gizzard shad can simultaneously influence zooplankton both directly (i.e. predation) and indirectly (via their effects on phytoplankton). Although increased planktivore abundance typically leads to improved resources for zooplankton (Drenner et al. 1984; Lazzaro 1987), the direct and indirect effects of larval gizzard shad on zooplankton eliminated any indirect positive effects for phytoplankton.

Although peak larval gizzard shad densities in Kokosing Lake appear high relative to published values (e.g. 20–25 fish/m³,

Mayhew 1977; 10 fish/m³, Downey and Toetz 1983; ~4 fish/m³, Matthews 1984; 0.5 fish/m³, Tisa et al. 1985), final densities in the experiment (6.0–15.5 fish/m³), all of which produced zooplankton crashes, lie within published ranges. Crustacean zooplankton in “exclosures” with fish densities <6 fish/m³ did not crash; their densities at experiment’s end were 96 organisms/L with 5.5 fish/m³ and 493 organisms/L with 1.5 fish/m³. Consequently, at least in Kokosing Lake, gizzard shad densities must exceed this threshold to drive zooplankton to extinction (as is the case for the yellow perch (*Perca flavescens*) – *Daphnia* interaction, Mills et al. 1987). Variations in year-class strength of gizzard shad coupled with complex interactions among young-of-year gizzard shad, zooplankton, and phytoplankton yield fluctuations in gizzard shad abundance. These fluctuations around the threshold density necessary for a zooplankton decline may lead to system-wide effects and certainly contribute to the tremendous system variability so characteristic of small reservoirs (e.g. see papers in Iowa Conservation Commission and Sport Fishing Institute 1983).

Effects on Larval Bluegill

Over a 4-yr span, gizzard shad and bluegill densities were inversely related. Given that appropriate-sized prey of a minimum density are required for larval fish survival (e.g. see Lasker 1975; Noble 1975; Werner and Blaxter 1980; Mills and Forney 1981), exploitative competition by gizzard shad might reduce prey to levels below that required by bluegill. Furthermore, slow growth could extend the period during which larvae are vulnerable to gape-limited predators (Rice et al. 1987a, 1987b). In either case, this inverse relation probably depends on relative spawning times. If bluegill spawned 2–4 wk earlier, their presence in the limnetic zone would occur during peak crustacean zooplankton abundance, perhaps leading to coexistence with gizzard shad. Conversely, if gizzard shad spawned 2–4 wk earlier, they would arrive in the limnetic before crustacean zooplankton peaked, possibly reducing gizzard shad success, as well as gizzard shad’s impact on zooplankton and larval bluegill.

Despite the dramatic impact of larval gizzard shad on zooplankton, diet overlap between bluegill and gizzard shad was not high. During 1987, overlap values increased during May through June as crustacean zooplankton became sparse and rotifers dominated diets of both species; this pattern did not occur during 1988 when bluegill and gizzard shad co-occurred on five sampling dates. Because bluegill spawned after gizzard shad in Kokosing Lake, gizzard shad always were larger than bluegill and, as such, ate a diversity of prey items as compared with first-feeding bluegill, which were restricted to copepod nauplii and rotifers (as in Mallin et al. 1985). Thus, gizzard shad appear to influence bluegill via exploitative competition (as opposed to interference competition between shad and white crappie, as in Guest et al. 1990), driving crustacean zooplankton to low levels before bluegill began exogenous feeding.

Competition between gizzard shad and bluegill occurs even though adult diets do not typically overlap, i.e. gizzard shad eat detritus (Dendy 1946; Pahl and Maurer 1962; Baker and Schmitz 1971; Mundahl and Wissing 1987) whereas bluegill eat zooplankton and littoral invertebrates (Keast 1978; Mittelbach 1984). In fact, coexistence between these species may be facilitated by the storage effect (Chesson and Warner 1981) because adult bluegill persist during those years when larval gizzard shad density is high and bluegill recruitment is low. However, interactions between young of these species have dra-

matic consequences for coexistence as in bluegill–pumpkinseed (*Lepomis gibbosus*) interactions (Werner and Gilliam 1984; Mittelbach and Chesson 1987; Mittelbach 1988). As we include larval and juvenile life stages in our exploration of aquatic communities, more complex interactions such as these will likely be discovered.

Historically, abiotic factors have been emphasized relative to control of recruitment in fishes (Busch et al. 1975; Clady and Hutchinson 1975; Clady 1976; Beam 1983). However, recent work suggests that biotic factors contribute substantially to recruitment variability (Lasker 1975; Forney 1976; Werner and Blaxter 1980; Rice et al. 1987a, 1987b; Post and Prankovics 1987). In Kokosing Lake, 87% of the year-to-year variability in bluegill recruitment was explained by variation in a biotic factor: peak larval gizzard shad density. In reality, several biotic mechanisms operate simultaneously through gizzard shad to influence bluegill recruitment, including direct competition (via predation on zooplankton) and indirect effects on zooplankton (see previous text).

The consequences of interaction between young-of-year gizzard shad and bluegill extend beyond bluegill recruitment, particularly because both gizzard shad and bluegill are forage species for piscivores (DeVries and Stein 1990). Gizzard shad recruitment varies independently of adult stock (Stock 1971), producing, we would argue, annual variation in zooplankton abundance, gizzard shad growth rates, and bluegill recruitment. Two factors that influence the susceptibility of prey to piscivores, bluegill recruitment and gizzard shad growth rates, vary with gizzard shad density, producing annual fluctuations in availability of prey to young-of-year largemouth bass. Because overwinter survival of young-of-year largemouth bass depends on body size and fat reserves in the fall (Adams et al. 1982), variations in prey availability ultimately will affect predator recruitment (see DeVries et al. 1991). Variation in recruitment alters adult population size, which, because fish are long-lived, will influence the aquatic community for many years, even decades in the future (Kitchell et al. 1988).

Implications for the Tropic Cascade

Gizzard shad are not controlled by fish predators owing to impressive fecundity (Bodola 1966; Wilder and Vondracek 1988; Parrish and Vondracek 1989), coupled with a narrow window of vulnerability to predators (Tisa et al. 1985; Johnson et al. 1988). Nor are they controlled by their zooplankton prey, for when zooplankton crash, gizzard shad switch to detritus and phytoplankton. We believe, therefore, rather than *being regulated* by top-down or bottom-up forces, gizzard shad regulate community composition via “middle-out” processes. In Ohio reservoirs, gizzard shad drive complex interactions among fish (by reducing bluegill abundance, and hence largemouth bass recruitment, via intense planktivory as young-of-year, DeVries et al. 1991), crustacean zooplankton (virtually eliminating it by midsummer in some years), and phytoplankton (directly by herbivory and indirectly by controlling zooplankton and nutrient limitation). As opportunistic omnivores that dramatically influence community composition via middle-out activities, gizzard shad requires reservoir food-web interactions in a fashion inconsistent with the trophic cascade paradigm so characteristic of north temperate systems.

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A Comparison of von Bertalanffy and Polynomial Functions in Modelling Fish Growth Data

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We compared the von Bertalanffy growth function (VBGF) and five polynomial functions (PF) in modelling fish growth for 16 populations comprising six species of freshwater fishes. Ranked results of the variance explained by each growth function indicated that VBGF described growth data better than three- and four-parameter polynomial functions. Log-transforming length and age greatly improved the goodness-of-fit of the three-parameter polynomial function. Statistical comparison of growth between populations or sexes was done using a general linear model for polynomial functions. An analysis of residual sum of squares was proposed to compare the resultant VBGFs because the nonlinear formulation of the VBGF prevented traditional analysis of covariance procedures. Fitting of different growth functions to the same growth data set yielded the same result in the intra-species growth comparisons for three species (eight populations) but different results for two species (seven populations). Where ages of the fish were less than the maximum age in the samples, dL/dt were similar for all growth functions except the parabola based on the log-transformation of length alone. The VBGF proved to be the best growth model for all 16 populations.

Nous avons comparé la fonction de croissance fondée sur l'équation de von Bertalanffy et cinq fonctions polynomiales appliquées à la modélisation de la croissance des poissons dans le cas de 16 populations (six espèces) de poissons dulcicoles. D'après le classement des résultats de la variance par rapport à chaque fonction de croissance, la fonction de croissance reposant sur l'équation de von Bertalanffy fournit un meilleur tableau des données sur la croissance que les fonctions polynomiales à trois et à quatre paramètres. La transformation logarithmique des données sur la longueur et l'âge a grandement amélioré la qualité d'application de la fonction polynomiale à trois paramètres. Nous avons procédé à une comparaison statistique de la croissance entre les populations ou les sexes au moyen d'une modélisation linéaire générale pour les fonctions polynomiales. Nous avons proposé d'analyser la somme des carrés obtenue, dans le but de comparer les résultats reposant sur l'équation de von Bertalanffy, car la formulation non linéaire de ce type de fonction nous empêchait de faire l'analyse de covariance classique. En comparant les données sur la croissance à l'intérieur d'une même espèce, l'application de diverses fonctions de croissance au même ensemble de données a abouti aux mêmes conclusions chez trois espèces (huit populations), mais les résultats différaient chez deux autres espèces (sept populations). Lorsque l'âge des poissons était inférieur à l'âge maximal des échantillons, les rapports dL/dt étaient semblables pour toutes les fonctions de croissance, exception faite de la parabole représentant la transformation logarithmique de la longueur seulement. La fonction de croissance fondée sur l'équation de von Bertalanffy s'est avérée le meilleur modèle de croissance des 16 populations étudiées.

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Fish growth data are usually fitted by an appropriate mathematical function to generalize the growth process, predict the growth trend, and compare the growth patterns between populations or species (Rao 1958; Moreau 1987). Of the mathematical functions applied to fish growth, the von Bertalanffy growth function (VBGF) (Ricker 1975; Pauly 1979; Moreau 1987) is used most widely by fisheries scientists. However, polynomial functions (PF) are being used with increasing frequency (e.g. Rafail 1972; Geoghegan and Chittenden 1982; Standard and Chittenden 1984; Chen et al. 1988), and they have been suggested to replace the VBGF in describing fish growth (e.g. Knight 1968; Roff 1980). Nevertheless, in recognition of the fact that even equivalent growth functions can produce divergent predictions of fish growth behaviour, it may be misleading to choose a function a priori without systematic comparison. Unfortunately, few such comparisons have been made between VBGF and PFs.

Fitting different growth functions to the same growth data set can yield different results and interpretations in the com-

parison of fish growth. For this reason, statistical comparisons are important when modelling fish growth data. However, despite the importance of testing the concordance between different mathematical functions fitted to the same growth data, few studies have considered this issue.

In this study, based on the growth data of six fish species differing greatly in growth, VBGF and five PFs were compared with respect to (1) goodness-of-fit, (2) the suitability and concordance of the statistical test, (3) reliability of coefficient estimates in the growth functions, and (4) the underlying implication of the growth functions in terms of growth rate in length as dL/dt .

Materials and Methods

Six fish species were collected from eight Ontario lakes and one Manitoba lake for this study (Table 1). Age of white sucker (*Catostomus commersoni*) was determined using the finray method (Beamish and Harvey 1969). Scales were employed to

TABLE 1. Information on species included in this study.

Species	Population	Sexed	Sample size	Plotted in Fig. 1
Pike	Wellman	Yes	31	Females
Pumpkinseed	Crosson	No	155	No
	Plastic	No	146	Yes
	Heeney	No	153	No
Rock bass	Plastic	No	181	Yes
Walleye	Wellman	Yes	174	Females
White sucker	Barry	Yes	41	Females
	Bentshoe	Yes	86	No
	Crosson	Yes	108	No
	Dickie	Yes	165	No
	McQuaby	Yes	92	Females
	Red Chalk	Yes	133	No
Yellow perch	Crosson	No	149	No
	Plastic	No	44	No
	Heeney	No	103	No
	Wellman	No	125	Yes

TABLE 2. Growth functions compared in this study.

Name	Mathematical expression
VBGF	$L_t = L_\infty \cdot (1 - \exp(-K \cdot (t - t_0)))$
PF 1	$L_t = a + b \cdot t + c \cdot t^2$
PF 2	$L_t = a + b \cdot \log_{10}(t+1) + c \cdot (\log_{10}(t+1))^2$
PF 3	$\log_{10} L_t = a + b \cdot \log_{10}(t+1) + c \cdot (\log_{10}(t+1))^2$
PF 4	$\log_{10} L_t = a + b \cdot t + c \cdot t^2$
PF 5	$L_t = a + b \cdot t + c \cdot t^2 + d \cdot t^3$

derive the age data for pumpkinseed (*Lepomis gibbosus*), yellow perch (*Perca flavescens*), rock bass (*Ambloplites rupestris*), and walleye (*Stizostedion vitreum*). Northern pike (*Esox lucius*) ages were based on both opercular bones and scales.

The VBGF is expressed as $L_t = L_\infty \cdot (1 - \exp(-K \cdot (t - t_0)))$, in which L_t is length-at-age, L_∞ is the asymptotic length, K is Brody growth coefficient, and t_0 is the age at which length is 0 (Ricker 1975). Standard nonlinear optimized techniques of curve fitting were used to estimate the coefficients and their associated standard error (Gallucci and Quinn 1979; Vaughan and Kanciruk 1982; Cerrato 1990).

A general polynomial function is usually expressed as:

$$f(x) = a + b \cdot x + c \cdot x^2 + \dots + k \cdot x^k \quad (k \text{ not equal to } 0)$$

in which $f(x)$ is a PF of degree (or order) n in the variable x . In particular, quadratic and cubic functions are the forms usually employed in fisheries (e.g. Knight 1968; Rafail 1972; Roff 1980). Four three-parameter polynomial functions were derived for this study (Table 2). In PF 2 and PF 3, t was replaced by $t + 1$ prior to a logarithmic transformation in order to calculate dL/dt for all ages. A four-parameter polynomial function was also included in this study (Table 2).

Because lengths-at-age of fishes at different age classes were derived from different sample sizes, data were weighted by the sample size to accurately represent length data for different age groups. To standardize the goodness-of-fit comparison, fitting of both PFs and VBGF was done using nonlinear optimization methods (Gauss-Newton method, NLIN of SAS Institute 1985). The measure of goodness-of-fit chosen was r^2 .

The r^2 values were ranked among the different growth functions for each population with the largest r^2 as 1, second largest as 2, and so on. A nonparametric test (Friedman 1937, 1940) was employed on the ranked results of r^2 to test the significance of the goodness-of-fit comparisons among growth functions. Friedman's test statistic, χ_r^2 , was calculated with a correction for tied ranks (Zar 1984). The association of rank ordering of r^2 values among growth functions was measured nonparametrically by the Kendall coefficient of concordance, ω (Kendall and Babington-Smith 1939; Kendall 1962). The distribution of χ_r^2 was considered to approximate the χ^2 distribution with $a - 1$ degrees of freedom. For this study, factors of the test (i.e. a) equal the number of the selected functions whereas blocks are the number of fish populations (Zar 1984). Pairwise comparisons of the goodness-of-fit also were made between growth functions.

Because of the nonlinear formulation of the VBGF, a general linear model could not be used for an analysis of covariance (ANCOVA). Instead, an analysis of the residual sum of squares (ARSS) was employed to compare VBGF between the sexes and among populations (Ratkowsky 1983). Procedures of the ARSS were as follows: (1) residual sum of squares (RSS) and an associated degree of freedom (DF) of VBGF were calculated for each sample, (2) the resultant RSS and DF of each sample were added to yield summed RSS and DF, (3) data of all samples were pooled to calculate the RSS and DF of a total VBGF, and (4) the F -statistic was calculated as

$$F = \frac{\frac{RSS_p - RSS_s}{DF_{RSS_p} - DF_{RSS_s}}}{\frac{RSS_s}{DF_{RSS_s}}} = \frac{\frac{RSS_p - RSS_s}{3 \cdot (K - 1)}}{\frac{RSS_s}{N - 3 \cdot K}}$$

where RSS_p = RSS of each VBGF fitted by pooled growth data, RSS_s = sum of the RSS of each VBGF fitted to growth data for each individual sample, N = total sample size, and K = number of samples in the comparison. This method was modified from the ARSS developed for the comparison between linear models (Zar 1984). To test whether there was a difference

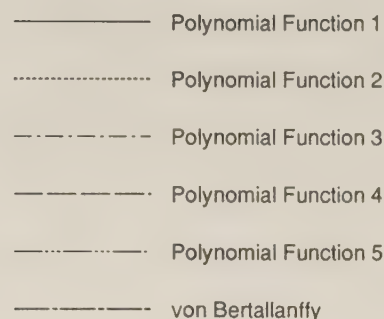
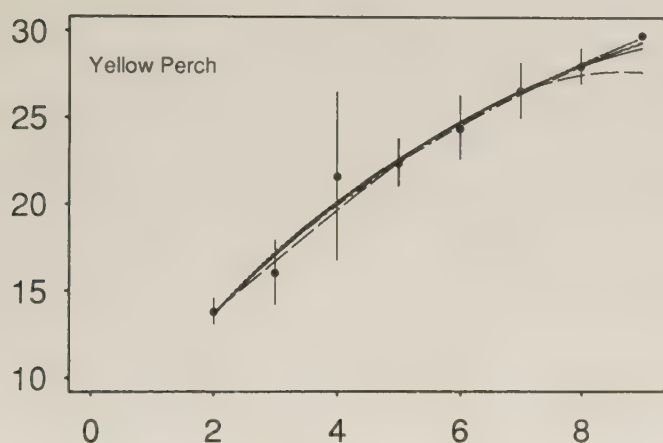
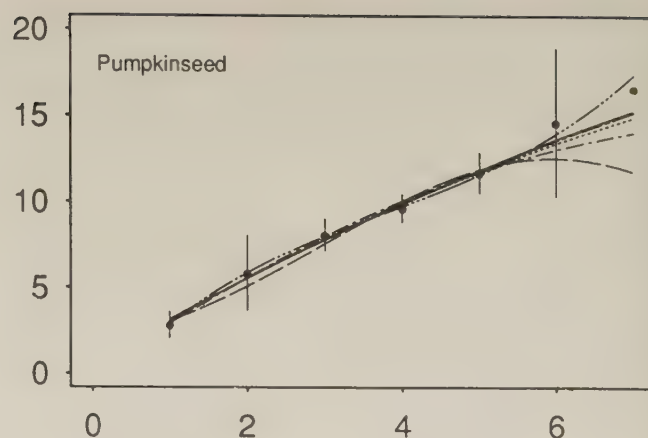
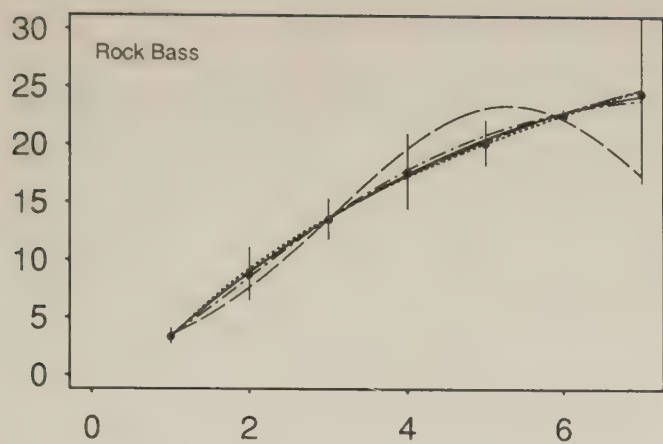


FIG. 1. Length-at-age curves for six fish species with the tested mathematical functions applied. Abscissa, fish age (yr); ordinate, fish fork length (cm); vertical bars, standard errors. (Fig. 1 concluded next page)

TABLE 3. Sum of r^2 ranks for each growth function. Numbers in parentheses are average ranks which equal the ratio of the sum of ranks over number of fish populations or sexually differentiated populations.

Species	VBGF	PF 1	PF 2	PF 3	PF 4	PF 5
Northern pike	3 (1.50)	10 (5.00)	7 (3.50)	6.5 (3.25)	12 (6.00)	3.5 (1.75)
Pumpkinseed	4 (1.33)	15 (5.00)	10 (3.33)	10 (3.33)	18 (6.00)	6 (2.00)
Rock bass	1 (1.00)	3 (3.00)	5 (5.00)	4 (4.00)	6 (6.00)	2 (2.00)
Walleye	3 (1.50)	10 (5.00)	10 (5.00)	4 (2.00)	10 (5.00)	5 (2.50)
White sucker	22 (1.83)	53.5 (4.46)	47 (3.92)	28.5 (2.38)	70 (5.83)	31 (2.58)
Yellow perch	6 (1.50)	18 (4.50)	16 (4.00)	15 (3.75)	20 (5.00)	9 (2.25)
Sum	39 (1.63)	109.5 (4.56)	95 (3.96)	68 (2.83)	136 (5.67)	56.5 (2.35)

in VBGF between the samples, the calculated F value was then compared with the critical F , with the DFs of numerator and denominator equal to $3 \cdot (K - 1)$ and $N - 3 \cdot K$, respectively. The ANCOVA was used for the PFs between the sexes and among populations (PROC GLM) of SAS Institute 1985). The results of the statistical comparison using ARSS and ANCOVA on VBGF and PFs were compared to determine whether there

were substantial differences in growth associated with the use of these different growth functions.

Parameters of each growth function were tested for all populations to find how many were not significantly different from 0. The growth rate in length as dL/dt is an underlying consequence of a growth function. Examination of differences in dL/dt between growth functions may help to explain the dif-

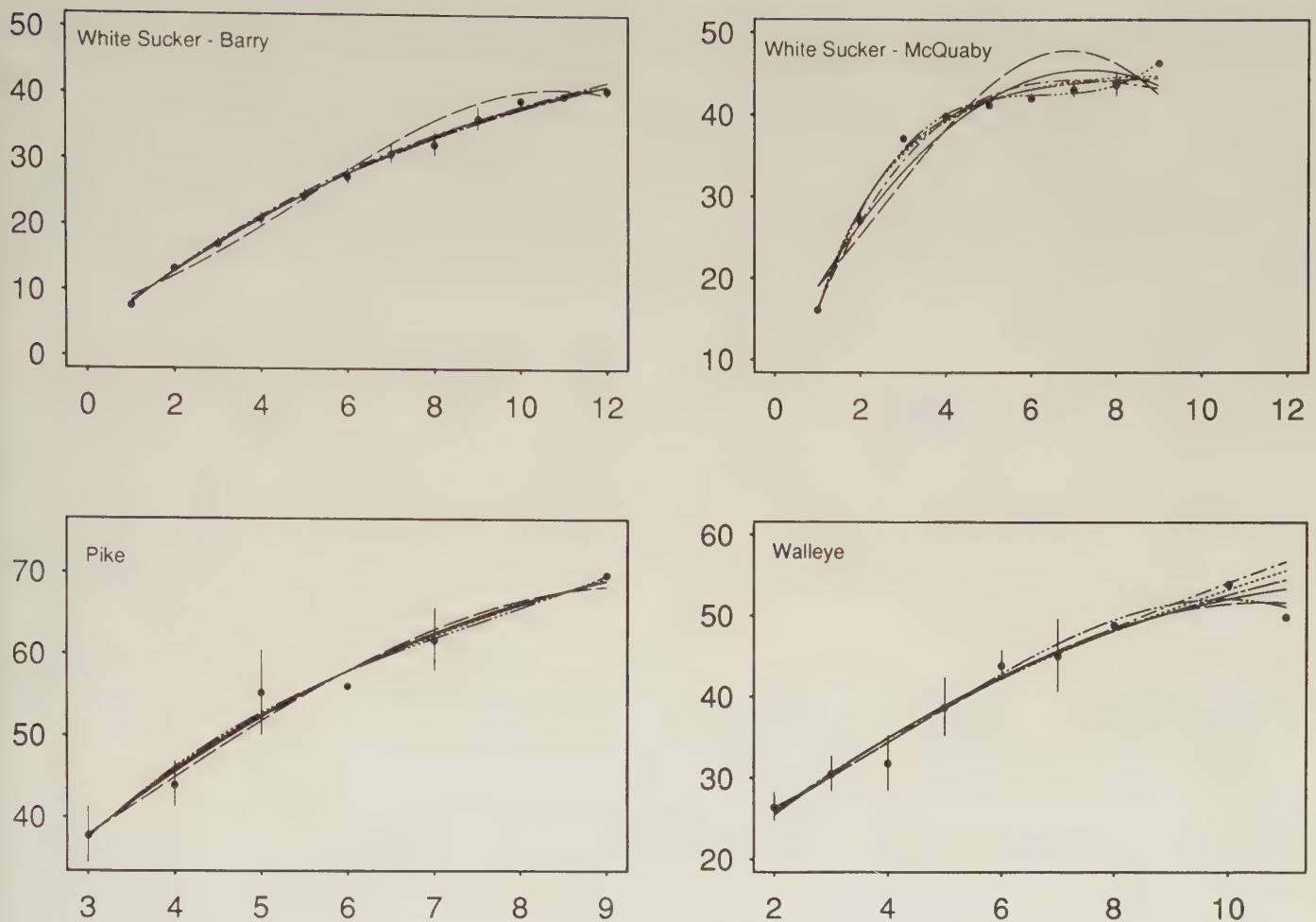


FIG. 1. (Concluded)

TABLE 4. Friedman's χ^2 and Kendall's coefficient of concordance, ω , for the pair comparisons between functions. Numbers above and below the diagonal are Friedman's χ^2 and Kendall's ω , respectively. *The function from the column heading has a significantly better ranking of r^2 values than the function identified in the corresponding row; **the function from the row heading has a significantly better ranking of r^2 values than the function identified in the corresponding column.

Function	VBGF	PF 1	PF 2	PF 3	PF 4	PF 5
VBGF		20.2**	16.7**	8.2**	24.0**	6.0
PF 1	0.8403		2.7	16.7*	16.7**	24.0*
PF 2	0.6944	0.2500		4.2	10.7**	10.7*
PF 3	0.3403	0.6944	0.1750		20.2**	0.7
PF 4	1.0000	0.6944	0.4458	0.8403		20.2*
PF 5	0.1736	1.0000	0.4458	0.0292	0.8403	

ferences in goodness-of-fit between these functions. The dL/dt derived from the different growth functions were examined using growth data of two white sucker populations with the high and low growth rates. Length-at-age and six fitted functions (VBGF plus five PFs) were plotted for populations of each species to illustrate the variations in growth among the six fish species.

Results

Goodness-of-Fit Comparison

A plot of length-at-age and six fitted growth functions for populations of each species (Table 1) showed that there were

great differences in growth among fish species (Fig. 1). The VBGF had smallest r^2 ranks for all six species (Table 3). The magnitudes in r^2 ranks in decreasing order were VBGF, PF 5, PF 3, PF 2, PF 1, and PF 4. Considering the tied rank groups in each block, Friedman's χ^2 statistic is 77.7 ($n = 24$). This was significant at the 5% level (i.e. $\chi^2 = 11.07$, $DF = 5$), indicating a significant difference in the rank ordering of the r^2 values among the growth functions. Kendall's coefficient of concordance was 0.648 ($n = 24$), indicating good agreement in the r^2 ranks for the different populations (i.e. blocks).

Based on paired comparisons between the VBGF and each PF, the VBGF was significantly better than PFs in goodness-of-fit (Tables 3 and 4). However, there were multiple comparisons here, and the selected significance level should be

adjusted. According to the Bonferroni method, significance level of 0.05 was divided by number of the functions involved in the comparisons, equalling 0.0083 (Miller 1977). The calculated level of significance between VBGF versus PF 5 was 0.0159 (Table 4), greater than the required adjusted p value of 0.0083. Therefore, there were no significant differences between the VBGF versus PF 5. Similarly, there were no significant differences in the goodness-of-fit between PF 5 and PF 3 ($p > 0.5$). However, PF 5 was significantly better than PF 1 ($p < 0.001$), PF 2 ($p = 0.001$), and PF 4 ($p < 0.001$) (Table 4). PF 3 fitted the growth data significantly better than PF 1 ($p < 0.001$) and PF 4 ($p < 0.001$), but had no significant difference with PF 2 ($p = 0.045$, which was greater than the required significance level using a Bonferroni adjustment, i.e. $p = 0.0083$). There were no significant differences in r^2 ranks between PF 1 and PF 2, but both fit significantly better than PF 4 (Tables 3 and 4). Consequently, in terms of the goodness-of-fit, VBGF was best, PF 3 and PF 5 were second best, and PF 4 was the worst for the growth data of this study (Tables 3 and 4). The better fit provided by PF 5 relative to other PFs is not unexpected given that PF 5 contains a third-order term not present in other PFs.

Suitability and Concordance of the Statistical Test

ARSS on the VBGF indicated that growth of white sucker from six populations differed significantly between the sexes and among populations (Table 5). There were significant differences in growth modelled by the VBGFs among the populations of pumpkinseed and among the populations of yellow perch (Table 5). No significant differences in VBGFs were found between the sexes for walleye and northern pike (Table 5).

Statistical analysis for the polynomial growth functions was done using ANCOVA to determine whether significant differences in growth existed between the sexes or among populations. The ANCOVAs for each of the five PFs indicated that there were significant differences in growth of white sucker between the sexes. Significant differences also were found among populations for both females and males. For pumpkinseed, the ANCOVAs indicated that there were significant differences among populations in growth modelled by PF 3, but not by the other four PFs. Significant differences among populations were not found in the growth of yellow perch modelled by PF 2, but were by the other four PFs. From the ANCOVAs on each of the PFs, it may be concluded that there were no significant differences between the sexes in the growth of both walleye and northern pike.

Reliability of Coefficient Estimates in the Growth Functions

Estimates of L_{∞} for the fishes in this study were close to the observed maximum length. The coefficient K in all resultant

VBGFs was between 0 and 1, and 16.7% of the estimates of t_0 were significantly greater than 0.

For polynomial growth functions, the number of samples with parameters significantly different from 0 was greater for the parabolas based on the log-transformation of length and both length and age (i.e. PF 3 and PF 4) than with the other PFs. For parameter d of the PF 5 (Table 2), 50% of the estimates were not significantly different from 0, indicating that the cubic factor in the function might not contribute substantially in fitting the growth data.

Implication of the Growth Functions

Female white sucker of the McQuaby population grew extremely rapidly when they were young (Fig. 2a). The growth rate in length as dL/dt was shown to be very similar among the growth functions except dL/dt derived from PF 4 between ages 2 and 8 (Fig. 2a). However, after age 8, only the VBGF described the growth rate as asymptotic to 0. For slow-growth females of the Barry population, dL/dt derived from all functions except PF 4 were similar until age 10, but after that, dL/dt of growth functions diverged. With the increase of age, VBGF tended to be the only function that could meaningfully describe the change of growth rate in length with age (Fig. 2b). The variability in dL/dt among growth functions was large for young fish (i.e. before age 2), especially in the McQuaby population with its high growth rate in length (Fig. 2a).

Discussion

Since the shape of the growth curve for fishes may vary between populations or species, the growth function that provides the best representation of growth data may vary also. Therefore, it is essential to assess the goodness-of-fit in any comparison among growth functions. The great difference in growth among these six species and the results of the comparison of goodness-of-fit indicate that the VBGF shows greater flexibility in describing growth data than the three- and four-parameter PFs. The three-parameter PF using log-transformed data for length-at-age and age explained the growth data better than those with log-transformation of only one variable or without logarithmic transformation (Tables 3 and 4). A four-parameter PF only described the growth data to a similar degree as the three-parameter PF with logarithmic transformation of length-at-age and age (Tables 3 and 4). However, when the significance level was adjusted according to the Bonferroni method (Miller 1977), differences in goodness-of-fit became nonsignificant between VBGF and PF 5. This result might suggest that although the VBGF is better than PF 5 in goodness-of-fit (Table 3), the differences between them were not great (Table 4). However, there are four parameters being estimated in PF 5, more than the parameters (i.e. three) in VBGF. It is

TABLE 5. Species comparison of growth modelled by VBGF by means of RSS. Rock bass was unsexed and had only one population available; thus, no statistical comparison was done for it.

Species	Comparison between	RSS _p	DF	RSS _s	DF	F	Pr > F
Northern pike	Sexes	110.55	9	105.53	6	0.10	>0.5
Pumpkinseed	Populations	206.86	16	30.39	10	9.68	0.0011
Walleye	Sexes	330.29	18	323.85	15	0.10	>0.5
White sucker	Populations (F)	32761.9	71	980.99	56	121.0	<0.0001
	Populations (M)	22588.8	66	221.18	51	343.8	<0.0001
	Sexes	59747.1	140	55350.7	137	3.63	0.0161
Yellow perch	Populations	1830.8	31	293.8	25	12.79	<0.0001

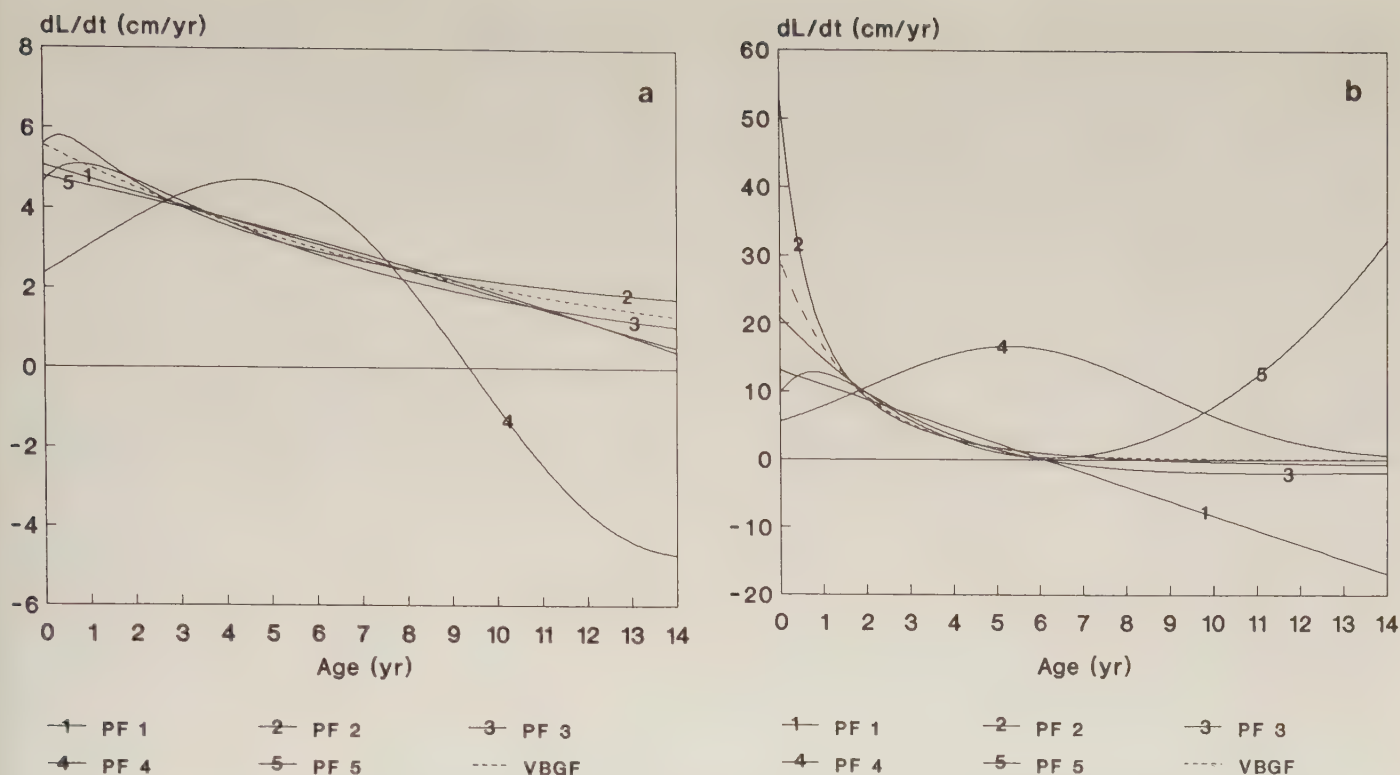


FIG. 2. Growth rate in length as dL/dt for female white sucker in (a) Barry Lake and (b) McQuaby Lake.

well known that there is a better chance of explaining more variance if more parameters are included in the function. If VBGF was compared with the PFs having the same number of parameters (i.e. three), it remained significantly better in goodness-of-fit after adjusting the level of the significance. Log-transformation of data is able to make the data more similar in magnitude, and perhaps reduces data-effect error (i.e. residual SS; Kimura 1990). Also, since there were no significant differences between PF 3 and PF 5, when a PF is used for fitting growth data, PF 3 may be the best choice in terms of goodness-of-fit.

Commonly used linear methods allowing statistical comparison of growth data (i.e. ANCOVA or ANOVA) cannot be employed on the VBGF because of its nonlinear formulation and high degree of correlation between its three parameters (Kingsley 1979; Gallucci and Quinn 1979; Misra 1980; Cerrato 1990). Two general approaches are usually suggested for the growth comparison of the VBGF. One is to test individual parameters (Gallucci and Quinn 1979; Kingsley 1979; Pauly 1979; Misra 1980; Bernard 1981). The other is based on likelihood ratio statistics (Kimura 1980; Kirkwood 1983; Cerrato 1990). However, there are many theoretical and practical problems in employing these two approaches (see Moreau 1987; Cerrato 1990). ARSS is informative and easy to calculate and for simplicity was used in the comparison of growth modelled by a VBGF.

Statistical comparisons for both PFs (based on ANCOVA) and VBGF (based on ARSS) showed that there were significant differences between the sexes and among populations for six populations of white sucker (Table 3). There were also no substantial differences in growth comparison for white sucker or between the sexes for both northern pike and walleye using PFs or VBGF. However, in the comparisons of pumpkinseed growth from three populations, substantially different conclusions were

drawn from using different functions. Significant differences in growth were found among populations if the growth data were modelled by the VBGF and PF 3, but the differences in growth were not found if other PFs (i.e. PF 1, 2, 4, or 5) were used. This function-selection-related difference in the results of growth comparisons was also observed for yellow perch. If growth data of yellow perch were fitted to PF 2, ANCOVA indicated that there were no significant differences among populations. However, if other PFs or VBGF were employed, significant differences were found by using the ANCOVA or ARSS. Thus, it is apparent that selection of different functions in describing fish growth may affect the results in growth comparisons (i.e. the power of the test between populations/sexes is influenced by the choice of growth functions). Recognizing this, caution is warranted in selecting a mathematical expression for fish growth data if the ultimate purpose of setting up a growth model is to compare growth between samples. We suggest that statistical comparisons be done for each of the functions selected.

Kohn (1986) stressed that if parameter values were estimated by formal optimization methods, it was essential to show that these estimates were reasonable and reliable for the biological data that the model was explaining. Therefore, in keeping with this view of the estimates of VBGF parameters, L_{∞} should be reasonably close to the maximum fish length in the sample (Taylor 1958; Pauly 1979; Moreau 1987), t_0 should be smaller than 0 so that fish at age 0 could have a positive length (Moreau 1987), and K might vary between 0 and 1 for fishes with long life spans (Pauly 1978). However, for PFs, there are no such criteria for judging the acceptability of the parameter estimates. Advocates of the PFs emphasize their mathematical simplicity rather than their biological usefulness.

Small differences between estimates of L_{∞} and observed maximum length indicated that the estimated L_{∞} values were

reasonable (Taylor 1958; Pauly 1979; Moreau 1987). Coefficient K in resultant VBGFs ranged from 0 to 1, indicating that these VBGFs were probably reasonable in describing growth data. Although 16.7% of the resultant t_0 estimates were significantly greater than 0, the exact value of t_0 is not usually considered to be important or necessary for some methods using t_0 , such as (1) yield per recruit computation, (2) use of length-converted catch curves for Z evaluations, and (3) length-based cohort analysis (Moreau 1987).

Large standard errors associated with the parameters may limit the ability to detect a significant difference between the parameters and 0, thus reducing the power of the tests. Those factors (i.e. t , t^2 , etc.) with parameters not significantly different from 0 were neither essential nor useful in describing the variance of the dependent variable (i.e. L_t). The number of parameter estimates significantly different from 0 in VBGF and PF 3 was substantially more than that in other PFs. This suggests that VBGF and parabolas with log-transformation of both length and age were better than other PFs in terms of reliability of the estimates of function parameters.

The general process of fish growth (where it is considered impossible that fishes will have negative growth rate in length) is well known; thus, it is more sensible to use the first derivative of growth functions (dL/dt) than growth functions themselves to judge whether the functions are reasonable in describing fish growth dynamics. dL/dt derived from PF 1 represents a straight line, meaning the growth rate in length changed constantly with age t . This pattern of growth rate might be true in the early life of fish or for short-lived fish (e.g. Nikolskii 1969; Standard and Chittenden 1984) but is not true for growth patterns of the entire life of long-lived fish (e.g. Nikolskii 1969; Kozlowski and Uchmanski 1987; Hayes and Taylor 1990). dL/dt derived from other PFs is parabolic curves related, indicating that there is a maximum or minimum dL/dt . Thus, if the growth process of fish is described by this function, two growth stages are included: increase and decrease in growth rate. This type of growth pattern often occurs in weight growth of fishes rather than in length growth (Nikolskii 1969).

Growth rates asymptotic to 0 are thought to accurately represent the growth of long-lived fish (e.g. Nikolskii 1969; Kozlowski and Uchmanski 1987; Chen et al. 1988; Murphy and Taylor 1989). Mathematically, it is obvious that no PF can describe this process. The plots of dL/dt on age for the two white sucker populations clearly illustrate this condition (Fig. 2), showing great differences between the trajectories of dL/dt derived from the PF 4 versus other functions. However, these differences are not great in the plot of L_t on age (Fig. 1). This indicates that differences in dL/dt trajectory are more apparent than those in length-at-age trajectory between growth functions. Therefore, plotting dL/dt may simplify the detection of differences between growth functions relative to the differences displayed in plots of L_t alone (Fig. 1 and 2).

The rate of growth in length as dL/dt derived from the VBGF realistically defined the patterns of fish growth in length. Based on the nonparametric analysis of r^2 ranks, the VBGF described the growth data better than three- and four-parameter PFs in this study. Before selecting the growth function for a growth data set, a systematic comparison should be done among growth functions being considered for use.

We suggest that VBGF be selected as the most suitable growth function for the six species in this study. If PFs must be chosen, we suggest the parabola with log-transformation of both dependent and independent variables.

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Response of a Subarctic Lake Chain to Reduced Sewage Loading

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Changes in total phosphorus (TP), chlorophyll *a* (Chl *a*), and total nitrogen (TN) concentrations were monitored in a lake chain in northern Quebec to which sewage loading had been reduced by about 80% over 8 yr. Summer concentrations of TP and Chl *a* fell by 70 and 78%, respectively, in Lake Pearce, the uppermost lake most affected by eutrophication. Reductions in the two downstream lakes were 64 and 55% for TP and 45% for Chl *a*. The rapid response of the lakes to reduced sewage loading is related to the short residence time of water (60 d in winter to 2 d during snowmelt in Lake Pearce). Sediments in Lake Pearce showed thick (20–70 cm) accumulations of soft, organic-rich material (loss on ignition 10–50%), containing 4–20 mg P·g⁻¹; however, winter P release rates were small (about 0.2 mg P·m⁻²·d⁻¹), possibly associated with the Fe-rich nature of the sediments. Although anoxic conditions still develop under the thick ice cover (1 m), the depth and intensity are less than before sewage reduction. Reductions in TP have been accompanied by increases in TN and TN:TP ratios (from <10:1 to >30:1), suggesting that there has been a shift from N to P limitation.

On a étudié les variations de concentration en phosphore total (PT), en chlorophylle *a* (Chl *a*) et en azote total (NT) dans un groupe de lacs situés dans le Nord québécois, dont la charge d'eaux usées a diminué d'environ 80 % en 8 ans. En été, les concentrations en PT et en Chl *a* ont diminué de 70 et de 78 %, respectivement, dans le lac Pearce, situé le plus en amont et le plus touché par l'eutrophisation. La baisse de concentration du PT dans les deux lacs situés en aval était de 64 et de 55 %, et celle de la Chl *a* atteignait 45 %. Le fait que les lacs ont réagi rapidement à la diminution de la charge d'eaux usées est lié au court temps de séjour de l'eau (dans le lac Pearce, c'est-à-dire 60 d en hiver et 2 d à la fonte des neiges). Les sédiments du lac Pearce étaient constitués d'une importante accumulation (20 à 70 cm) de matières organiques molles (perte par calcination de 10 à 50 %), contenant de 4 à 20 mg P·g⁻¹; en hiver, cependant, les taux de libération du P étaient faibles (environ 0,2 mg P·m⁻²·d⁻¹), la diminution enregistrée pouvant être imputable à la haute teneur en fer des sédiments. Bien qu'il existe encore des conditions d'anoxie sous l'épaisse couche de glace (1 m), la profondeur touchée et la sévérité de l'anoxie sont moindres qu'avant la diminution des déversements d'eaux usées. Les baisses de PT ont été accompagnées d'une hausse de l'azote total et du rapport NT : PT (de <10 : 1 à >30 : 1), ce qui semble indiquer que la limitation du N enregistrée auparavant a fait place à une limitation du P.

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Eutrophication processes resulting from increased nutrient inputs into lakes are reasonably well understood, but much less is known about responses to reduced inputs of nutrients, an increasingly common phenomenon (Levine and Schindler 1989). Lake response to reduced nutrient loading is dependent on a number of factors, the most important of which are likely to be lake morphometry, hydrology, and thermal regime and the ability of the lake sediments to take up or release nutrients.

Several studies have documented the response of lakes to reduced nutrient loading, at scales ranging from experimental mesocosms (e.g. Levine and Schindler 1989) to small lakes (e.g. Ahlgren 1972, 1978a, 1978b; Larsen et al. 1975; Michal-ski et al. 1975) and large lakes (e.g. Edmondson 1970, 1972; Edmondson and Lehman 1981; Lean 1987; Lean et al. 1990; Stevens and Neilson 1987). Attention has generally been focused on changes in concentrations of nitrogen and phosphorus, which are believed to be the controlling nutrients, dissolved oxygen profiles, and biological attributes, such as chlorophyll concentration. Marsden (1989) has recently reviewed results of lake restoration studies, particularly the role

of a reduction in external loading of nutrients, such as phosphorus, and the release of this element from lake sediment.

In this paper, we report our observations of changes in total nitrogen (TN), total phosphorus (TP), chlorophyll *a* (Chl *a*), dissolved oxygen (DO) concentrations, and Secchi disc depths in a chain of three small subarctic lakes located near Schefferville, northern Québec. For about 20 yr, Lake Pearce, the uppermost number of the chain, received the untreated sewage from the town of Schefferville, whose population ranged from 2000 to 4000. Drake and Freund (1980) studied the fate of sewage-added P in the lake chain in 1975 and estimated that approximately 80% of the input sedimented in Lake Pearce. In 1975, a primary sewage treatment plant was introduced, which probably reduced the loading of P to Lake Pearce by about 20% (Drake and Freund 1980). A further reduction in nutrient loading occurred when the town's population dropped to about 600, with closure of the iron mines in 1983. Using data collected in 1975 (Freund 1977) and during the 1980s, we trace the effects of reduced sewage input on the chemistry and biology of the lakes in the chain.

Lake Descriptions

The lake chain is fed by Lake Knob (Fig. 1), which also provides drinking water to Schefferville and has been relatively unaffected by disturbance. Lakes Pearce, June, and LaCosa are small, shallow lakes with large catchment areas (Table 1). Lakes in the Schefferville area generally are oligotrophic in character, although those underlain by dolomite can be classified as mesotrophic. TP and Chl *a* concentrations measured in six lakes of the Schefferville region over several years ranged from 1 to 10 and from 0.5 to 3.5 $\mu\text{g}\cdot\text{L}^{-1}$, respectively (O. Choulik and F. H. Rigler, unpubl. data; Smith et al. 1984a, 1984b).

The lake catchments contain spruce-lichen woodlands, spruce-moss forests, and fens, which dominate the Schefferville area (Waterway et al. 1984). The soils are shallow (<1 m), derived from Fe-rich sedimentary and metamorphic rocks of the Labrador Trough. They are generally acid and low in available nutrients, such as N and P (Moore 1980).

The winters are long and cold and the summers cool, with January and July mean air temperatures of -23 and 12°C , respectively. The mean annual precipitation is 791 mm, of which 383 mm falls as snow. The lakes are generally ice-free from June 15 to October 15, with 1 m of ice developing during

the winter. They are weakly or not thermally stratified during the summer because of their shallowness and northwest-southeast alignment parallel to the dominant wind direction. During the winter, an inverse thermal stratification develops. Snow-melt dominates the hydrology of the lakes; Drake and Freund (1980) estimated a mean residence time of water in Lake Pearce of about 60 d during the winter whereas it was about 2 d during the peak of melt in May. With an annual runoff of about 500 mm, Lake Pearce receives an amount of water each year equivalent to about 10 times its volume.

Materials and Methods

Nutrients and Secchi Disc

Water samples were taken from three locations in each lake (Fig. 1) at approximately 2-wk intervals from mid-June to mid-September 1982–90. At each location, a 2-cm-diameter tube was lowered down through the water to just above the sediments and a depth-integrated sample was collected. TP, TN, and Chl *a* concentrations measured in these samples were used to compute whole-lake values. Secchi disc depth was also measured at each location.

TP was analyzed by the persulphate-mixed reagent method (Strickland and Parsons 1968). Potassium persulphate (0.4 g)

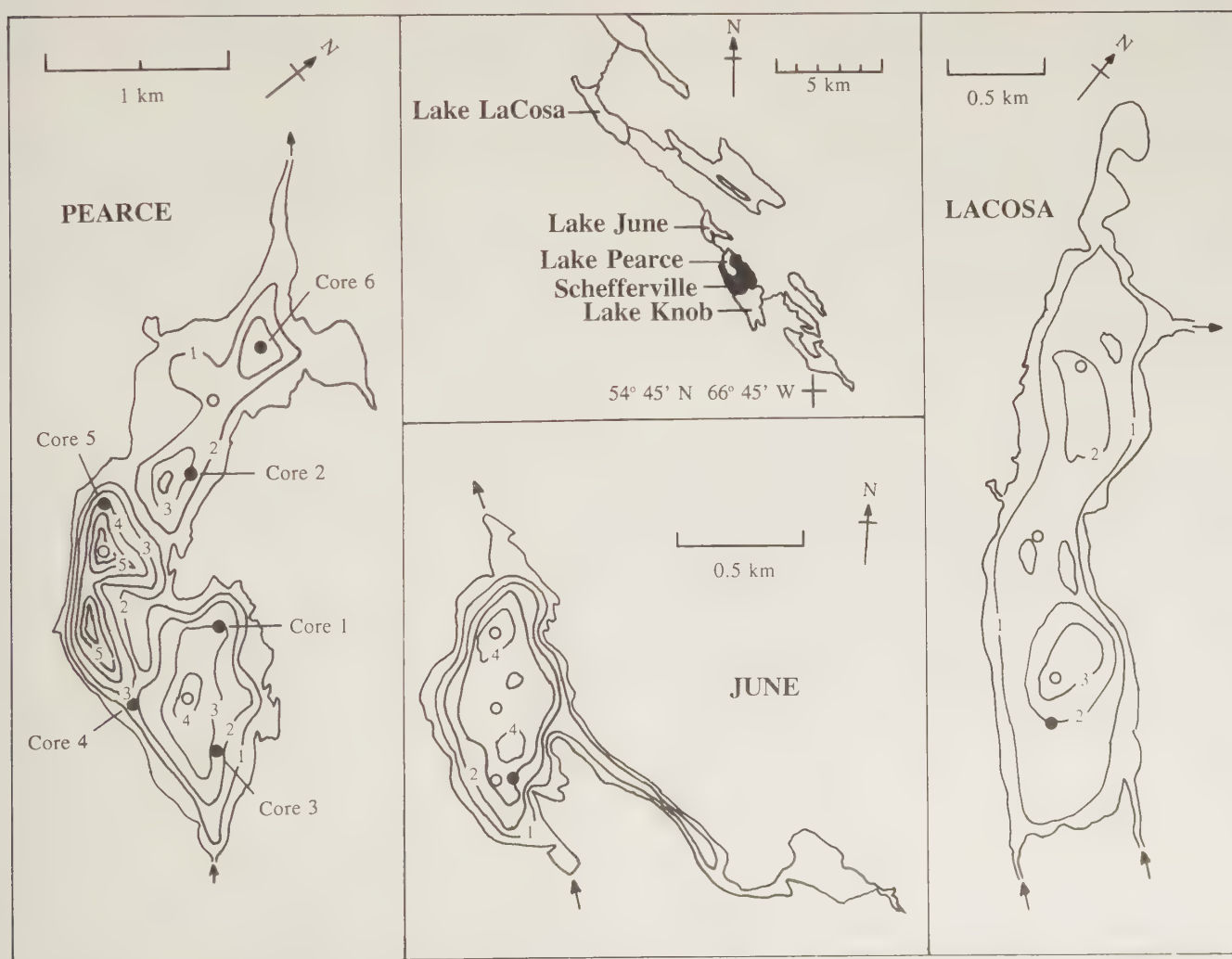


FIG. 1. Location and characteristics of the lake chain. Bathymetric survey contours in metres. Open circles indicate the sites used for the lake water sampling and closed circles the location of the sediment cores.

TABLE 1. Characteristics of the three lakes in the chain.

Lake	Catchment area (km ²)	Lake area (km ²)	Mean depth (m)	Volume (m ³ × 10 ⁶)
Pearce	36	0.56	2.3	1.7
June	60	0.45	2.3	1.1
LaCosa	195	2.13	1.6	3.5

was added to 50 mL of lake water and autoclaved at a pressure of 1 kg·cm⁻² for 1 h. Six millilitres of mixed reagent (ascorbic acid – ammonium molybdate – sulfuric acid – potassium antimonyl tartrate) was added to the cold samples and absorbance read on a Bausch and Lomb Spectronic 100 spectrophotometer at a wavelength of 885 nm.

For TN analysis, 0.2 g of potassium persulphate and 0.4 mL of 6 N NaOH were added to 30 mL of lake water and autoclaved for 1 h. Five millilitres of the solution was added to 2 mL of sodium salicylate, evaporated to dryness, and 1 mL of concentrated sulfuric acid added to dissolve the precipitate. After the addition of 30 mL of distilled water, 5 mL of Rochelle salts was added and the absorbance read at a wavelength of 420 nm (Kalff and Bentzen 1984).

Chl *a* was measured by filtering 1 L of lake water through a Gelman A/E glass fibre filter. After extraction in the dark with 95% ethanol for 1–3 d, the samples were filtered and absorbance read at 649, 665, and 750 nm. Chl *a* was calculated from the difference in absorbance at 665 and 649 nm after correction for turbidity at 750 nm (Smith et al. 1984a).

Winter DO and TP

During the winter of 1986–87, additional water samples were collected for DO analysis from the deepest location of Lake Pearce (maximum depth 6 m) using a peristaltic pump. Lake water was collected at 1-m depth intervals from beneath the ice to just above the sediment surface. DO was determined by the Winkler iodometric method (American Public Health Association 1976). Over the winter of 1988–89, similar samples taken from the same locations in Lake Pearce were analyzed for TP as described above.

Sediment Cores

Sediment cores from the four lakes (six in Lake Pearce and one each in Lakes Knob, June, and LaCosa) were collected in mid-June 1987 using 10-cm-diameter ABS tubes pushed into the lake floor. The core collected the organic surface sediment and down into the underlying red mineral sediment; core lengths ranged from 20 cm in Lake Knob to over 80 cm in Lake Pearce. The tubes were frozen and the core cut into 2-cm sections. After drying at 60°C, the samples were weighed to calculate bulk density, ground to pass a 2-mm sieve, and analyzed for loss on ignition at 550°C and TP by the ascorbic acid reduction method after digestion with boiling 1 N HCl (Andersen 1976).

Results

Changes in Nutrients and Chl *a*

Lake Pearce

Whole-lake TP concentrations in Lake Pearce decreased substantially from a mean of 76 µg·L⁻¹ in 1975 (Freund 1977) to 53 and 41 µg·L⁻¹ in 1982 and 1983 and further to between 13 and 31 µg·L⁻¹ from 1984 to 1990 (Table 2). The most pronounced decline was prior to 1984, the TP concentrations remaining relatively constant since then.

Chl *a* concentrations were 40 µg·L⁻¹ in 1967–68 (J. Kalff, unpubl. data), 15.3–17.2 µg·L⁻¹ in 1982–83, and between 6.4 and 8.7 µg·L⁻¹ during 1986–90. Secchi disc depth was 0.4 m in 1975, but increased to 1.1 m in 1982–83 and has remained at about that level since. TN concentrations were 0.19–0.37 mg·L⁻¹ during 1982–85 and increased to 0.92–1.16 mg·L⁻¹ in 1986–87 before falling back to 0.45 mg·L⁻¹ in 1990.

TN:TP ratios increased from 4.6–7.0 in 1982–83 to 46.4–48.4 in 1986–87 and 34.9 in 1990. Chl *a* : TN ratios decreased from 0.041–0.091 in 1982–83 to 0.007–0.0014 during 1986–90. In contrast, the Chl *a* : TP ratio changed little from a minimum of 0.28 in 1989 to a maximum of 0.56 in 1984.

Lake June

Concentrations of TP in Lake June decreased from a mean of 50 and 53 µg·L⁻¹ in 1975 and 1982, respectively, to 16–24 µg·L⁻¹ during 1987–90, with the most prominent decline before 1984 (Table 2). Chl *a* concentration fell from a mean of 14.7 µg·L⁻¹ in 1982 to 6.0–8.3 µg·L⁻¹ during 1986–90, with the greatest decline before 1984. Secchi disc depths increased slightly. TN concentrations were 0.29–0.30 mg·L⁻¹ in 1982 and 1984 and rose to over 1.0 mg·L⁻¹ in 1986 and 1987 before falling to 0.43 mg·L⁻¹ in 1990.

Accompanying these changes in Lake June was a rise in TN:TP ratios from 5.7 in 1982 to 27–65 during 1986–90, and the Chl *a* : TN ratio fell over this period from 0.049 to 0.007–0.014. The Chl *a* : TP ratio changed little over time, with an average value of 0.44.

Lake LaCosa

In 1975, Lake LaCosa had the lowest TP concentration of the three lakes (whole-lake mean of 25.3 µg·L⁻¹). TP concentrations fell to 9.2–16.1 µg·L⁻¹ during 1984–90 (Table 2). Concentrations of Chl *a* were within the range 2.3–4.8 µg·L⁻¹, and the Secchi disc depth ranged from 1.1 to 1.7 m. TN concentrations were low (0.15–0.27 mg·L⁻¹) during 1982–85 but rose to over 0.7 mg·L⁻¹ in 1986–87 before falling to 0.39 mg·L⁻¹ in 1990.

TN:TP ratios in Lake LaCosa rose from 7.9 in 1982 to 100.9 in 1987, and there was an accompanying fall in the Chl *a* : TN ratio from 0.032 to 0.005. The Chl *a* : TP ratio ranged from 0.26 to 0.50, with a mean value of 0.34.

The trajectories of Chl *a* : TP concentrations in the three lakes are plotted in Fig. 2 and reveal a strong and relatively constant relationship over the ranges encountered. The overall relationship between Chl *a* and TP concentrations can be expressed by the regression

$$\log_{10} [\text{Chl } a] = 0.936 \log_{10} [\text{TP}] - 0.338$$

$$r^2 = 0.801, p = 0.001, n = 23, \\ \text{standard error of estimate} = 0.121.$$

There was no significant relationship between TN and Chl *a* ($p = 0.70$).

Changes in DO

An anaerobic hypolimnion developed in Lake Pearce beneath an ice cover of 1 m during the 1986–87 winter (Fig. 3). DO concentrations were high directly beneath the ice cover, probably partly associated with the freeze-out of oxygen as the ice thickened. DO concentrations were reduced to <2 mg·L⁻¹ in the bottom 3 m of the 6-m-deep water column by March 1987, while the lower 2 m contained <1 mg·L⁻¹. This was followed

TABLE 2. Properties of the three lakes by year.

Year	TP ($\mu\text{g}\cdot\text{L}^{-1}$)	TN ($\mu\text{g}\cdot\text{L}^{-1}$)	Chl <i>a</i> ($\mu\text{g}\cdot\text{L}^{-1}$)	TN:TP	Chl <i>a</i> : TP	Chl <i>a</i> : TN	Secchi (m)
<i>Lake Pearce</i>							
1967–68	—	—	40	—	—	—	—
1975	76	—	40	—	0.53	—	0.4
1982	53	370	15.3	7.0	0.29	0.041	1.1
1983	41	190	17.2	4.6	0.42	0.091	1.1
1984	18	300	10.0	16.7	0.56	0.033	1.6
1985	31	410	11.4	13.2	0.37	0.028	1.3
1986	25	1160	8.4	46.4	0.34	0.007	1.4
1987	19	920	8.5	48.4	0.45	0.009	1.3
1989	31	—	8.7	—	0.28	—	1.1
1990	13	450	6.4	34.9	0.49	0.014	1.3
CV ^a	0.44	0.36	0.25	—	—	—	0.27
<i>Lake June</i>							
1967–68	—	—	—	—	—	—	—
1975	50	—	—	—	—	—	—
1982	53	0.30	14.7	5.7	0.28	0.049	1.1
1983	—	—	—	—	—	—	—
1984	12	0.29	8.7	23.4	0.71	0.031	1.5
1985	24	0.37	9.4	15.3	0.39	0.026	1.3
1986	21	1.10	8.3	51.6	0.39	0.008	1.5
1987	17	1.13	8.1	65.1	0.47	0.007	1.2
1989	24	—	8.3	—	0.35	—	1.2
1990	16	0.43	6.0	26.9	0.38	0.014	1.3
CV ^a	0.38	0.46	0.22	—	—	—	0.27
<i>Lake LaCosa</i>							
1967–68	—	—	—	—	—	—	—
1975	25	—	—	—	—	—	—
1982	19	0.15	4.8	7.9	0.26	0.032	1.7
1983	—	—	—	—	—	—	—
1984	11	0.27	4.4	24.5	0.40	0.016	1.4
1985	13	0.26	4.6	21.0	0.37	0.017	1.1
1986	12	0.72	3.8	61.6	0.32	0.005	1.4
1987	8	0.82	4.1	100.4	0.50	0.005	1.1
1989	16	—	4.2	—	0.26	—	1.2
1990	9	0.39	2.3	42.4	0.25	0.006	1.2
CV ^a	0.40	0.45	0.29	—	—	—	0.15

^aCV = Average seasonal coefficient of variation.

by aeration of the water column during the spring melt. The profile is similar to that found by Freund (1977), except that the anoxic zone in 1975–76 developed earlier, at the beginning of February, and rose 1 m higher in the water column.

An indication of the change in oxygen depletion within Lake Pearce can be derived from a comparison of the DO profiles determined by Freund (1977) over the 1974–75 winter and those obtained at the same site in 1985–86 (Fig. 4). In 1974–75, the lower 3.5 m of the water column contained $<2 \text{ mg DO}\cdot\text{L}^{-1}$ by early May, and the bottom 2.5 m had $<0.5 \text{ mg DO}\cdot\text{L}^{-1}$. In contrast, during the 1985–86 winter, there were higher DO concentrations on both the December–January and May sampling dates, with only the bottom 1 m of the column containing $<2 \text{ mg DO}\cdot\text{L}^{-1}$ in May 1986.

Sediment Characteristics and P Release

The six sediment cores collected from Lake Pearce revealed unconsolidated, black organic matter overlying red mineral material, representing the sewage and original mineral sediments, respectively. The thickness of the black material ranged

from 12 to 66 cm, with the greatest thickness in the southern section, close to the input of sewage (Table 3). Assuming that the change in sedimentation occurred soon after the founding of the town of Schefferville in 1954, this represents an average sewage–sediment accumulation rate of between 0.4 and $2.2 \text{ cm}\cdot\text{yr}^{-1}$. The sediment core collected from the central section of Lake June revealed only 8 cm of organic sediment over the red mineral substrate, and a poorly defined, 6 cm-thick organic layer was detected in the core from Lake LaCosa.

The different origins of the sediment material are reflected in analytical data for two of the cores from Lake Pearce (Fig. 5). Bulk density was generally $<0.1 \text{ g}\cdot\text{cm}^{-3}$ in the black organic layer, with a pronounced increase to values $>0.3 \text{ g}\cdot\text{cm}^{-3}$ in the lower mineral layer. Loss on ignition values also abruptly changed at this boundary, the black material having values between 10 and 50%. The greatest changes were in P content. In the red mineral matter, the P concentrations rarely exceed $1 \text{ mg}\cdot\text{g}^{-1}$ whereas in the black material, the concentrations were commonly $5 \text{ mg}\cdot\text{g}^{-1}$ and reached $18 \text{ mg}\cdot\text{g}^{-1}$ in some sections of the cores. Although the major reduction in sewage loading to the lake occurred 5 yr before the cores were col-

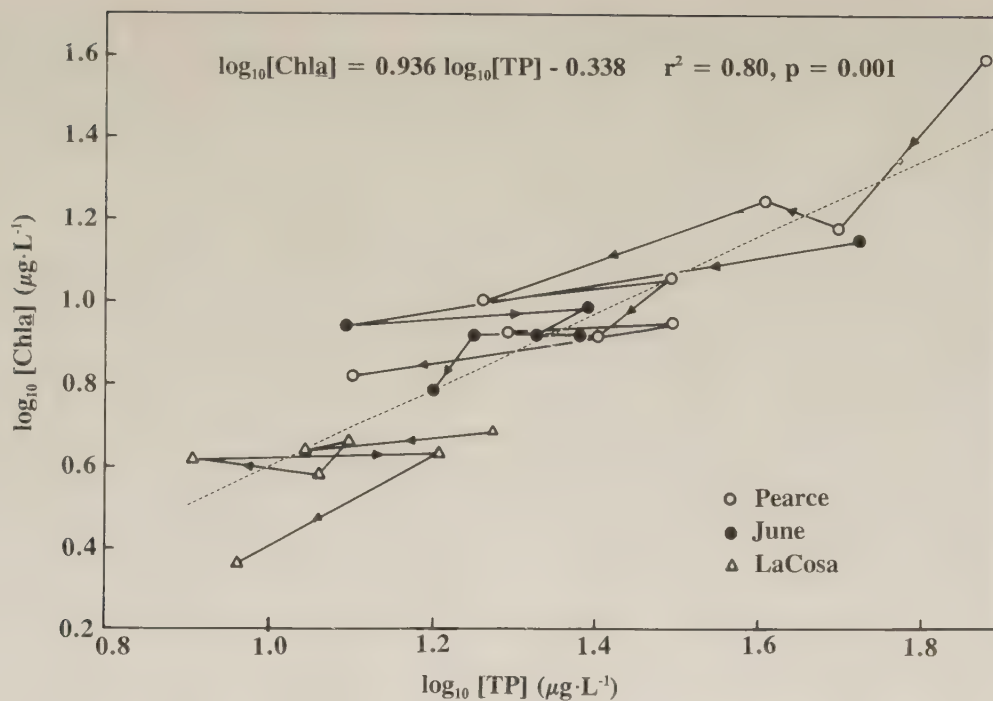


FIG. 2. Trajectories of Chl *a* : TP in the three lakes. The broken line represents the log:log regression.

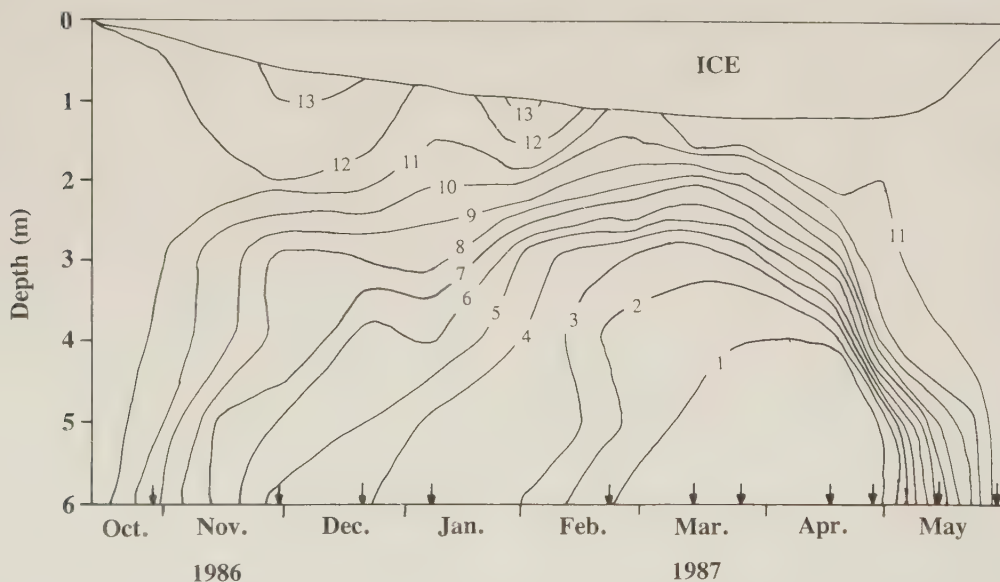


FIG. 3. DO in Lake Pearce, winter 1986-87. Samples from 1-m increments were collected on the dates indicated by the arrows.

lected, there was no evidence from any of the six cores for smaller sediment P concentrations in the surface sediments.

The same general pattern occurred in the core analyzed from Lake June (Fig. 6), although the enrichment of P to concentrations of $3 \text{ mg} \cdot \text{g}^{-1}$ occurred only in the top 8 cm and the organic layer was underlain by consolidated mineral matter with a high bulk density ($1.2\text{--}1.5 \text{ g} \cdot \text{cm}^{-3}$). The Lake LaCosa core revealed only very minor evidence of P enrichment in the upper 7 cm, with P concentrations $< 2 \text{ mg} \cdot \text{g}^{-1}$, although the whole core was less consolidated (bulk density values between 0.2 and $0.4 \text{ g} \cdot \text{cm}^{-3}$) and richer in organic matter (loss on ignition values of $10\text{--}15\%$) than the mineral layers in Lake Pearce.

An estimate of the amount of anthropogenic P accumulated in the lakes can be obtained from bulk density and P concentration in the section of the core above the boundary of organic

sedimentation, as shown by the red/black colour change and the increased concentration of P. From 0.06 to $0.45 \text{ kg P} \cdot \text{m}^{-2}$ accumulated above the red mineral layer in the six cores collected from Lake Pearce, with the highest values in the central and southern sections of the lake (Table 3; Fig. 1). The single core from Lake June had a P accumulation of $0.06 \text{ kg} \cdot \text{m}^{-2}$ in the organic top 8 cm, and accumulations in the cores from Lakes Knob and LaCosa were estimated to be 0.01 and $0.06 \text{ kg} \cdot \text{m}^{-2}$.

These results on the cores allow an estimate of the total amount of P accumulated in Lake Pearce since the development of the town of Schefferville, based on the mineral/organic boundary. Although there is high spatial variability in P accumulation, based on the variability among the cores taken from the lake, the average accumulation for the whole lake is likely

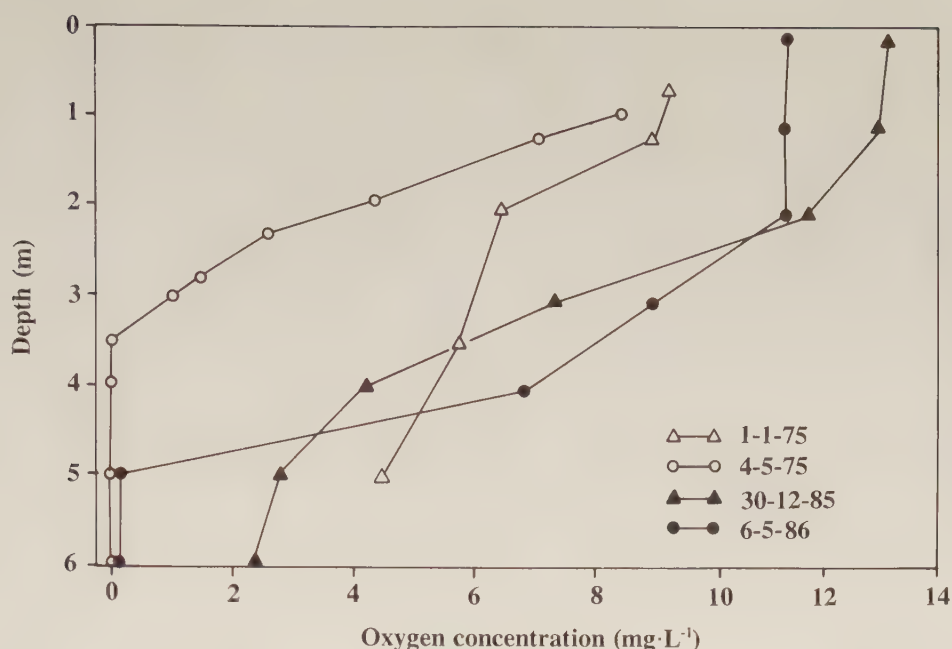


FIG. 4. DO profiles in Lake Pearce, January 1 and May 4, 1976 (Freund 1977), and December 30, 1985, and May 4, 1986.

TABLE 3. Depths of organic matter rich sediment in the cores collected from the three lakes and estimated P loading above the mineral layer.

Lake	Core number	Thickness of organic layer (cm)	Estimated P accumulation ($\text{kg} \cdot \text{m}^{-2}$)
Pearce	1	66	0.26
	2	22	0.17
	3	38	0.45
	4	16	0.20
	5	25	0.39
	6	12	0.07
June	1	8	0.06
LaCosa	1	6	0.06
Knob	1	2	0.01

to be in the range of $0.15\text{--}0.30 \text{ kg P} \cdot \text{m}^{-2}$. With an area of 0.56 km^2 , this amounts to a TP accumulation in Lake Pearce of between 84 and 168 t, with an average accumulation of between 3 and $6 \text{ t P} \cdot \text{yr}^{-1}$ over the 30 yr since the town was founded.

The deposition of a sediment rich in organic matter and P and the development of anoxic conditions suggest that there may be a substantial release of P from the sediments during the winter. This was tested by measuring the increase in TP contained in the Lake Pearce water column over six sampling dates, from December 1988 to May 1989 (Fig. 7). The average rate of TP increase in the lake was $0.1 \text{ mg P} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ over the 130-d period before snowmelt. However, it should be recalled that the residence time of water in Lake Pearce during the winter is about 60 d (Drake and Freund 1980), with water supplied from Lake Knob being low in TP. Thus, the release of P from the Lake Pearce sediments is likely to be greater than the value calculated, probably about $0.2 \text{ mg} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$.

Discussion

This subarctic lake chain has responded to a pronounced decrease in the external loading of nutrients, especially P con-

tained in sewage. This decrease is partly associated with the introduction of a primary sewage treatment plant in 1975. Drake and Freund (1980) estimated reduced sewage P loading to the lake of 20%, although we believe that the plant worked inefficiently in the following decade. The major decrease in P loading was achieved by the decrease in the population of Schefferville. At the peak of the iron mining activity in the 1960s and 1970s, between 3000 and 4000 people lived year-round in the town (Table 4), but this began to decrease as the mines reduced operations in the late 1970s and were closed in 1982 (Bradbury and St. Martin 1983). About 2500 people left for the south and the population was further decreased by the movement of about 500 Naskapi to a new village 20 km from the town in 1984. The current population of Schefferville is about 600, so there has been a reduction in population, and thus sewage loading to the lake, of about 80% in a decade, the most pronounced change occurring during 1979–83.

These changes in population and sewage treatment have reduced the loading of nutrients to the Lake Pearce system. Drake and Freund (1980) estimated that the loading to Lake Pearce in 1975 amounted to $7.3 \text{ t} \cdot \text{yr}^{-1}$, of which $6.6 \text{ t} \cdot \text{yr}^{-1}$ came from sewage and the remainder from the catchment, mainly Lake Knob. The anthropogenic loading has been reduced to between 0.5 and $1 \text{ t P} \cdot \text{yr}^{-1}$, based on either a prorating of the 1975 data for a decrease in population or by assuming a human P production of $0.8 \text{ kg} \cdot \text{capita}^{-1} \cdot \text{yr}^{-1}$ (Reckhow and Simpson 1980). Thus, at the present time, annual external loading of P to the lake is about equally distributed between natural and anthropogenic sources. A reduction in P loading of this magnitude has been reported in several other lakes (e.g. Marsden 1989).

The trophic status of Lake Pearce has decreased substantially. The mean summer concentration of TP during 1984–90 had fallen by 70% from 1975 concentrations and by 57% from 1982 concentrations. Chl *a* concentrations have fallen by 78 and 42% over the same period. The most pronounced declines occurred prior to 1984, after which there have been few significant changes. The lake is still mesotrophic, according to the

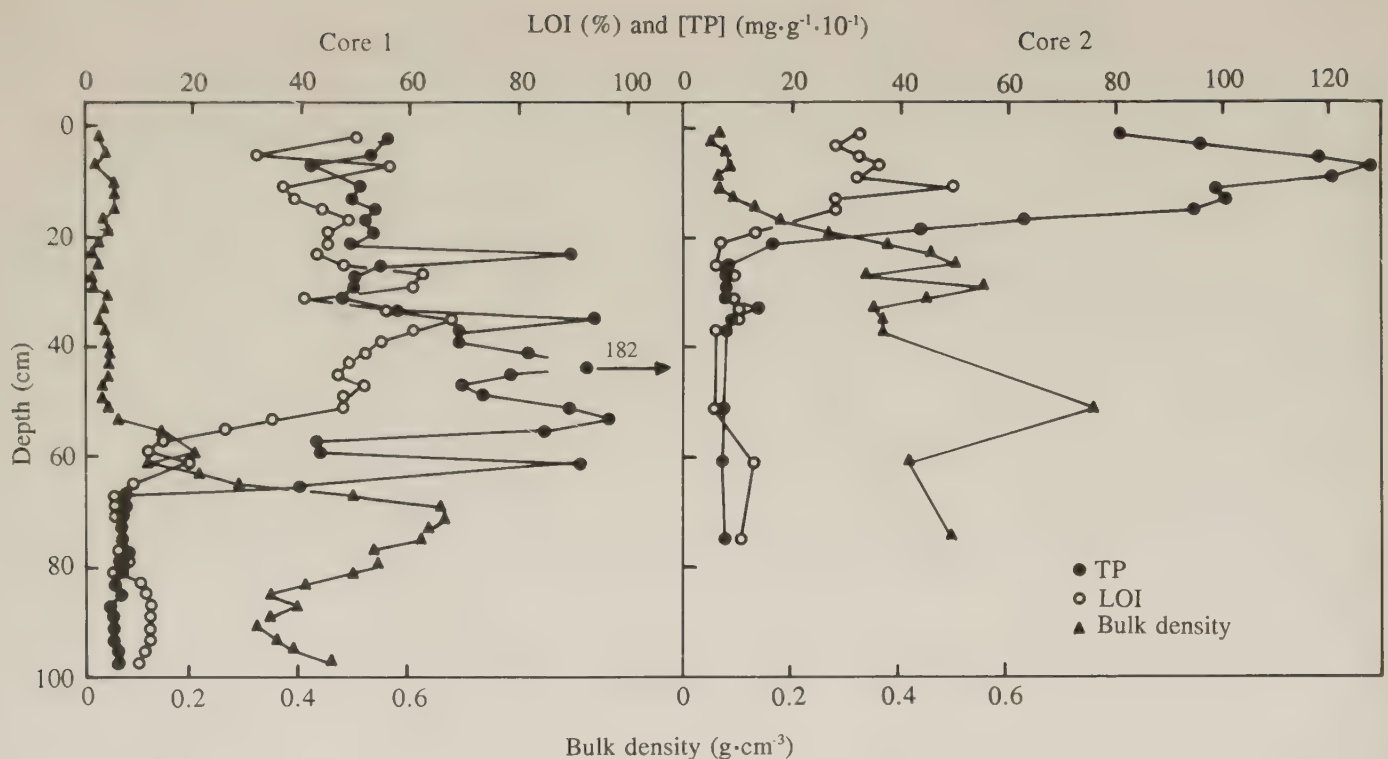


FIG. 5. Bulk density, loss on ignition, and TP concentration in two cores taken from Lake Pearce.

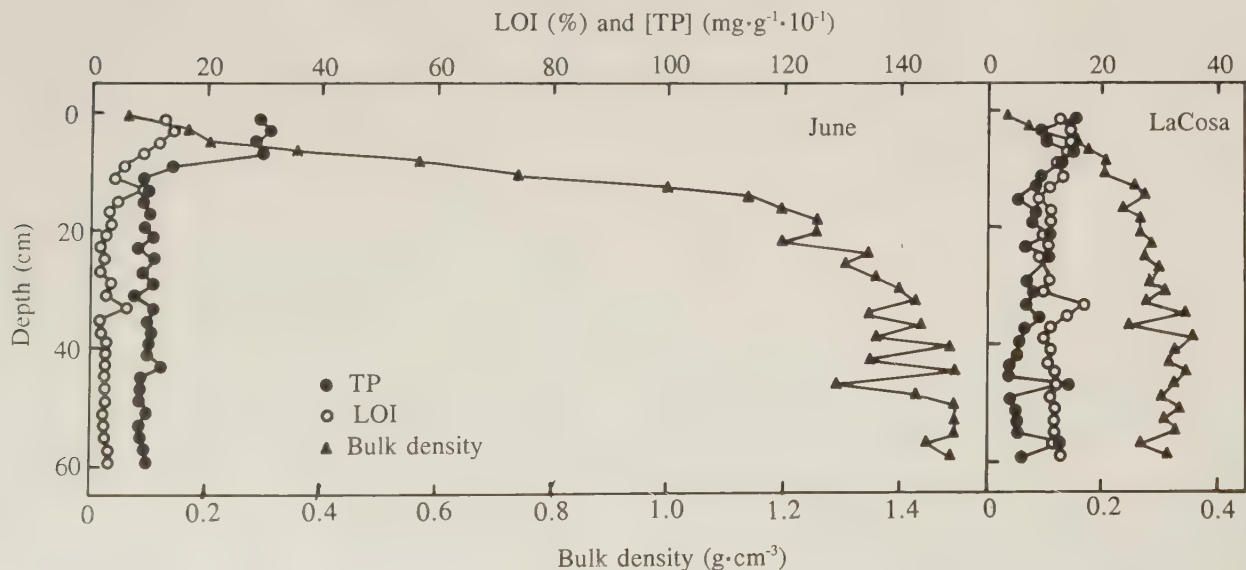


FIG. 6. Bulk density, loss on ignition, and TP concentration in cores taken from Lakes June and LaCosa.

definition of Wetzel (1983). Lake June, which received sewage effluent not trapped by Lake Pearce, has also experienced 64 and 45% reductions in summer TP and Chl *a* concentrations, respectively, over the same period, 1984–90 versus 1982. The third lake in the chain, Lake LaCosa, was the least eutrophic but there still has been a significant reduction of 55% in 1984–90 mean summer TP concentrations from the value in 1982. Absence of Chl *a* measurements in 1982 preclude a comparison with current concentrations.

The rapid and pronounced response of Lake Pearce to reduced P loading could be anticipated, as its storage capacity is small compared with annual runoff. Residence times for water in this

lake range from 2 d during snowmelt to 60 d during winter. Marsden (1989) noted that flushing rate controls the ability of a lake to respond to reductions in external loading and reduces the effect of internal loading from sediments. Reduction in external P loading to Lake Pearce has produced a response in TP concentration and biomass (Chl *a* concentration) similar to that observed in Lake Ennell, Ireland (Marsden 1989), Lake Gjersjoen, Sweden (Sas and Vermij 1987), Loch Leven, Scotland (Bailey-Watts 1982), and Lake Ekoln, Sweden (Ryding 1981). With a similar reduction in P loading and TP concentration, however, Lake Pearce showed stronger reductions in biomass than Lake Shagawa, USA (Larsen et al. 1975, 1981;

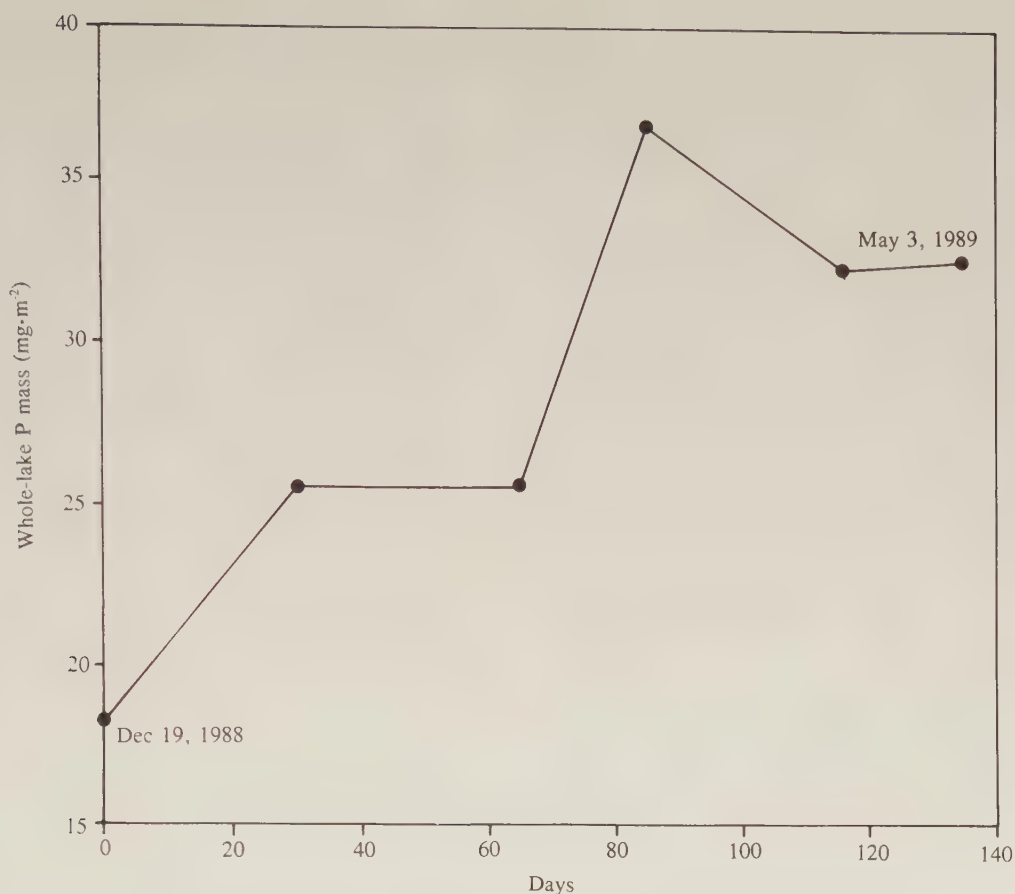


FIG. 7. Whole-lake mass of P in Lake Pearce, winter 1988–89.

TABLE 4. Estimated population of Schefferville contributing sewage to Lake Pearce, based on Census Survey and town estimates (Archer 1983).

Date	Population
1956	1632
1961	3178
1966	3086
1971	3275
1976	4025
1979	4129
1981	2700
1986	725
1989	600

Smith and Shapiro 1981). Lean et al. (1990) and Stevens and Neilson (1987) reported P loading and lake water P concentration decreases of about 40 and 60%, respectively, and a reduction in Chl *a* concentration of about 50% during 1967–87 in Lake Ontario.

The Schefferville lake chain is capable of high rates of P retention (Drake and Freund 1980), partly because of the Fe-rich nature of the rocks, soils, and groundwater in the Schefferville area. Phosphate adsorption on Fe hydroxides is a major mechanism for P retention in lakes (e.g. Manning 1987), and the soils and sediments of the Schefferville area contain up to 3% dithionite-extractable Fe and haematite and magnetite (Nicholson and Moore 1977). Using a mass balance approach, Drake and Freund (1980) estimated a P retention rate of 75% for Lake Pearce in 1975. The large amount of P stored in the recent, organic-rich sediments (up to 0.5 kg P·m⁻², with an

average of about 0.2 kg P·m⁻²) suggests that over the past 30 yr since the development of the town, about 100 t of P has been retained in Lake Pearce. Based on data from cores collected in 1975, Drake and Freund (1980) estimated that the annual P sedimentation in Pearce Lake was 5.0 t.

The estimated rate of P release from the sediments of Lake Pearce after the period of major sewage loading, determined by increases in TP concentration during the winter, is small compared with values obtained elsewhere. A daily rate of 0.2 mg·m⁻²·d⁻¹ and an annual release period into anoxic water of 75–80 d translates into an annual P release rate of 15–16 mg P·m⁻²·yr⁻¹. Nurnberg (1984) gathered internal P loading rates for a number of lakes. For oxic lakes, the internal P loading ranged from –1353 to 198 mg·m⁻²·yr⁻¹ whereas for anoxic lakes, the values ranged from –1080 to 4186 mg·m⁻²·yr⁻¹. For anoxic lakes after sewage diversion, the range was from 53 to 4200 mg·m⁻²·yr⁻¹, but with most values from 50 to 400 mg·m⁻²·yr⁻¹. Although the Lake Pearce sediments are rich in P, the release of P is limited by the short period of anoxic conditions, the low temperatures during the winter, and, possibly, the strong bonding between P and Fe minerals in the sediment. Manning (1987) and Nurnberg (1988) have suggested that release of P from sediments may be closely related to Fe minerals and the rate at which these minerals may be reduced.

Accompanying the decrease in TP and Chl *a* concentrations, there has also been an increase in the DO concentration in the Lake Pearce water column during the winter. Calculated winter oxygen depletion rate (WODR) for Lake Pearce is 54 and 24 mg O₂·m⁻²·d⁻¹ for 1974–75 and 1985–86, respectively, using whole-lake DO measurements. These calculated rates are much

lower than those obtained for other lakes in the Schefferville region (60–222 mg O₂·m⁻²·d⁻¹; F. H. Rigler and O. Choulik, unpubl. data) and for other northern lakes (e.g. Welch 1974; Welch and Bergmann 1985). The low calculated WODR for Lake Pearce may be related to the replenishment of oxygenated water from Lake Knob and groundwater.

The lack of significant change in Secchi disc depth measurements over the period reflects the shallow nature of the lakes and the disturbance of sediments by wind.

Through experimental additions of P and N to the lakes of the Schefferville region, Smith et al. (1984a, 1984b) showed that there was a very strong relationship between TP and Chl *a* concentrations, but that response of chlorophyll to P addition occurred only when N was added at the same time. Their (Smith et al. 1984b) overall regression between log₁₀[Chl *a*] and log₁₀[TP] has intercept (−0.559) and slope (0.975) values similar to those obtained in this study for the lake chain which has had reduced P loading. Recently, Prairie et al. (1989) calculated an overall regression for Chl *a* : TP with similar intercept (−0.390) and slope (0.874) values. However, they noted that the regression values changed as the TN:TP ratio varied in the lakes.

TN:TP ratios in the Schefferville lakes were <10 during the early years of the temporal sequence and rose to values >30 after reduced sewage loading. Based on critical TN:TP ratios of 15 for N and P nutrient limitation (OECD 1982), the lakes may have been N limited initially but have become P limited with reduced sewage loading. The increase in TN:TP ratios from <10 to >30 is partly dependent on smaller TP concentrations but also on greater TN concentrations. In Lake Ontario, Lean et al. (1990) have also reported a 50% increase in TN concentrations, a doubling of NO₃[−]-N concentrations, and a tripling of TN:TP ratios from 10:1 to 60:1, but primarily associated with increased atmospheric and fertilizer loading of N, rather than a response to decreased P loading. The mechanisms for increases in TN concentrations after sewage loading are unknown, and interactions among N, P, and biological response warrant further study (Elser et al. 1990).

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Morphology and Evolution of Salmonid Habitats in a Recently Deglaciaded River Basin, Washington State, USA¹

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Morphology and distribution of salmonid habitats were related to the geomorphology of a river basin at three spatial scales including reach (10^2 – 10^3 m²), subbasin (2–26 km²), and the watershed (240 km²). Stream reaches on a young fluvial terrace (1700 yr old) adjacent to the main river contain the most extensive areas of rearing and spawning habitats. In tributary subbasins, the area of spawning habitat varies according to discharge rates and channel gradients. The most extensive salmonid habitats are located along wide glacial deposits in geologically unconstrained areas of the main valley floor. During the early Holocene (~10 000 – 12 000 years before present (B.P.)), the recently deglaciaded watershed of the South Fork Stillaguamish River was extremely erosive and vegetated by alpine forest. Fish habitats then were less suitable for salmonid rearing and spawning. A much lower erosion rate after 8000 yr B.P., and the advent of old growth conifer forests after 6000 yr B.P., indicates that stream habitats attained their present-day morphology between 8000 and 6000 yr ago. Although habitats increased in quality with increasing watershed stability and evolution of forests, they decreased in quantity after 7000 yr B.P. as landforms changed because of continuous river incision into glacial deposits.

La morphologie et la répartition des habitats de salmonidés étaient liées à la géomorphologie du bassin fluvial en fonction de trois échelles spatiales, c'est-à-dire à l'échelle du tronçon (10^2 – 10^3 m²), à celle du sous-bassin (2–26 km²) et à celle du bassin hydrographique (240 km²). Les tronçons de cours d'eau sur les terrasses fluviales récentes (datant de 1 700 ans) adjacents à l'artère fluviale principale renferment les alevinières et les frayères les plus étendues. Dans les sous-bassins d'affluents, la superficie des frayères varie selon le débit et la pente d'écoulement du canal. Les habitats de salmonidés les plus vastes se trouvent le long de larges dépôts glaciaires dans des zones exemptes d'obstacles géologiques et situées au fond de la vallée principale. Au début de l'holocène (soit environ 10 000 à 12 000 ans avant le présent (B.P.)), le bassin hydrographique de la rivière South Fork Stillaguamish, dont la déglaciation était récente, subissait une très forte érosion, et ses berges étaient végétalisées par une forêt alpestre. À cette époque, les habitats de poissons étaient moins favorables à l'alevinage et à la fraye de salmonidés. Un taux d'érosion beaucoup plus faible depuis 8 000 ans B.P. et la présence de vieilles forêts de conifères depuis 6 000 ans B.P. indiquent que les habitats lotiques ont atteint leur morphologie actuelle il y a 6 000 à 8 000 ans. La qualité des habitats s'est accrue grâce à la stabilité plus grande du bassin hydrographique et à l'évolution des forêts; toutefois, le nombre d'habitats a diminué depuis 7 000 ans B.P., au fur et à mesure que le relief changeait et que les cours d'eau s'encaissaient toujours davantage dans les dépôts glaciaires.

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Fishery biologists, geomorphologists, and hydrologists in northwestern North America have made considerable progress in describing factors that influence the morphology of salmonid habitats in streams. Most researchers have considered habitats at the scale of reaches (e.g. pools and riffles (10^1 – 10^4 m²)). Pertinent examples include the role of organic debris in structuring pool habitats (Bisson et al. 1987), variation in the quality of spawning gravels due to channel aggra-

dation with fine or coarse sediment (Collings et al. 1972; Everest et al. 1987), and the utility of hydraulic geometry measurements in predicting usable habitat areas (Hogan and Church 1989).

Fish habitat has also been studied at the scale of entire watersheds in the Pacific Northwest. Cederholm and Reid (1987) combined an analysis of the distribution of coho salmon (*Oncorhynchus kisutch*) in stream habitats with an investigation on erosion processes to determine the effects of forestry activities on salmon production in the Clearwater River basin

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(375 km²), Washington State. Their work identified streams formed on fluvial terraces as important refuge habitats for winter rearing. Cederholm and Reid (1987) also suggested that sediment production from roads and landslides reduced the survival of eggs in the redd pockets.

At a smaller basin scale, Hartman et al. (1987) examined responses of various salmonid communities to several forestry activities in the Carnation Creek watershed (10 km²), British Columbia. As part of their work, Hartman et al. (1987) identified small, low-order tributaries as having an important effect on habitat of larger streams. The Hartman et al. (1987) and Cederholm and Reid (1987) studies demonstrated the importance of considering the entire watershed when studying the morphology of salmonid habitats and the effects of land use.

This study differs from previous habitat investigations by examining how geomorphic or land-forming processes facilitate or limit the development of salmonid habitat in tributary streams over long periods (10²–10³ yr). Such an analysis allows insight into how the development of salmonid habitats is linked to the formation of landscapes. The recently deglaciated environment of northwestern Washington State provides a unique opportunity to study the evolution of stream habitats over a period of approximately 14 000 yr.

We studied the present-day morphology and distribution of salmonid habitats in the South Fork Stillaguamish River valley in northwest Washington State at a variety of spatial scales ranging between reach (10²–10³ m²), tributary basin (2–26 km²), and the watershed (240 km²). Habitats were considered in the context of the geomorphic processes responsible for their morphology. Salmonid habitats included summer rearing habitat, defined as low-velocity pools with cover provided by organic debris or undercut banks, and spawning habitat, defined as clean gravels between 13 and 75 mm in diameter.

A hypothesis was developed on the evolution of stream habitats in the study watershed since continental deglaciation (~14 000 years before present (B.P.)). This hypothesis was based on the information on present-day morphology and distribution of habitats, a radiocarbon dating analysis of the erosional history of the basin, and published literature on the development of forests in the Pacific Northwest.

Study Area

The South Fork of the Stillaguamish River basin ("South Fork") is located in the Cascade Mountains of northwest Washington, USA (Fig. 1). The river basin (240 km²) contains 18 tributary basins with areas ranging from 2 to 26 km². Elevations of the river basin range from 300 m at the downstream end of the study area to over 1800 m in the eastern portion of the basin. Annual precipitation varies from about 305 cm at Verlot (U.S. Forest Service Visitor Center) to 450 cm at the summit of Mt. Pilchuck. Snow accumulates throughout the winter months at elevations greater than 500 m. The basin is vegetated with dense stands of Douglas fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), and true firs (*Abies* spp.), a forest community referred to as the Western Hemlock Zone (Franklin and Dyrness 1973). Most of the natural forests are referred to as "old growth," that is, much of the forest is composed of trees greater than 250 yr old.

The river basin is composed of a variety of lithologies including Jurassic Period (136–195 million yr) metamorphic rocks in the western portion and Tertiary Period (12–65 million yr) sedimentary and volcanic rocks in the eastern portion (Dugan 1974; Heath 1971; Wiebe 1963). The basin has been influenced by

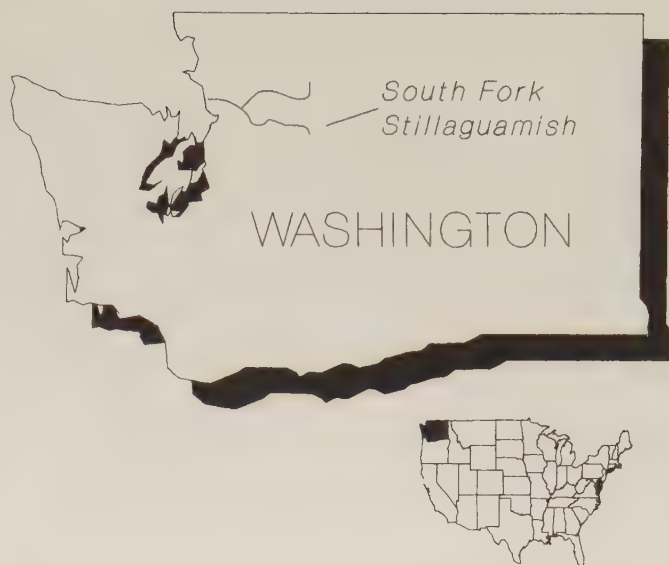


FIG. 1. Location of the South Fork of the Stillaguamish River basin.

continental glaciation throughout much of the Quaternary Period (last 2 million yr). Most recently, a several-thousand-metre-thick cordilleran ice sheet buried most of western Canada and the northwestern edge of the United States during the late Pleistocene Epoch. Glacial damming of the South Fork by the ice sheet resulted in thick deposits of lacustrine silt and clay in the valley (Fig. 2). Retreat of the ice sheet approximately 14 000 yr ago in northwestern Washington State (Crandell 1965; Mullineaux et al. 1965) also resulted in extensive deposition of sandy, glacial outwash sediments in the valley of the South Fork. Other surficial deposits include talus (rockfall) and alluvial/debris fans at mouths of tributary valleys at the contact between bedrock and glacial sediment (Fig. 2). The surficial deposits cover 65 km² (26% of the basin area) and extend in elevation from the valley bottom to approximately 600 m.

In recent history the upper 56 km of the South Fork was inaccessible to anadromous fishes because of a natural migration barrier at Granite Falls. Access was permitted by the construction of a fish ladder in 1954. Since then, coho salmon, chinook salmon (*Oncorhynchus tshawytscha*), pink salmon (*O. gorbuscha*), and steelhead trout (*O. mykiss*) have been introduced by hatchery releases above the falls. As of the late 1970s, coho and steelhead were well established above the falls based on number of returning adults (South Fork Stillaguamish River B.U.M.P. 1978). Resident salmonid species include cutthroat trout (*Oncorhynchus clarki*) and rainbow trout (*O. mykiss*).

Habitats of anadromous salmonids, primarily coho and steelhead, occur in low-gradient (<4%) reaches of tributary streams formed in glacial sediments. High-gradient (>4%) reaches of streams on glacial sediments and channels underlain by bedrock are too steep to support anadromous salmonids. However, they provide habitat for resident species such as rainbow and cutthroat trout.

The main channel of the South Fork provides limited spawning habitat because of the high proportion of cobble and boulder substrate but provides rearing habitat in boulder pocket pools and along the edges of large pools and glides. Snorkel surveys indicate that approximately 20–30% of total summer pool and glide surface area in the main river is suitable for coho rearing (J. Doyle, Mt. Baker-Snoqualmie National Forest, Supervisor's Office, Seattle, WA, pers. comm.).

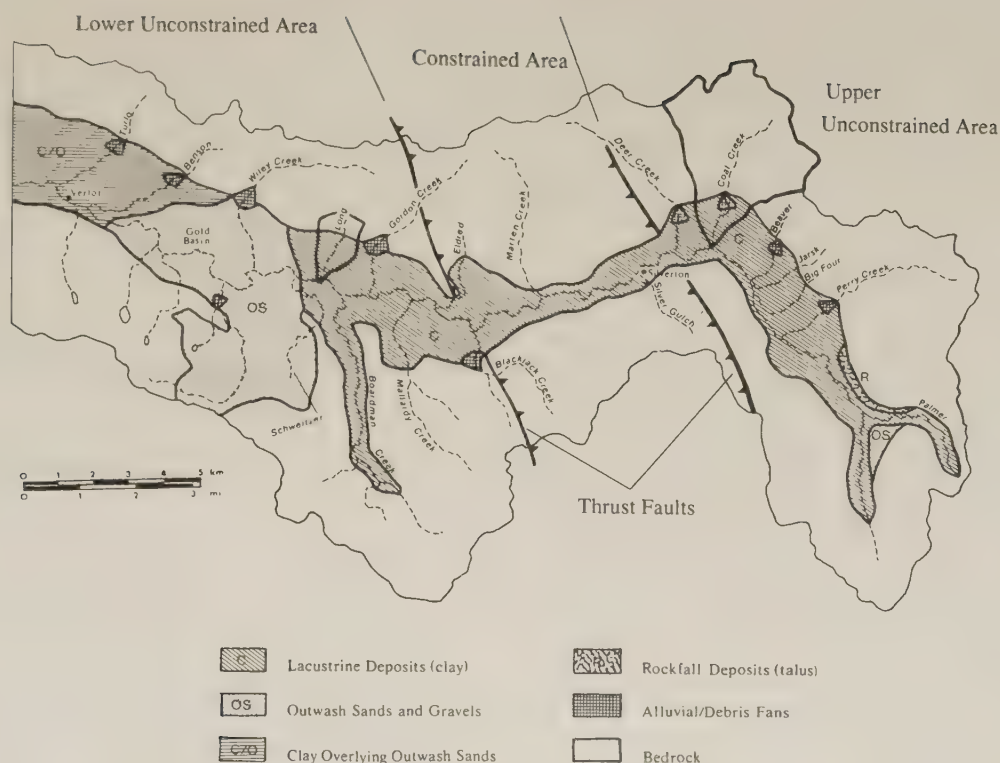


FIG. 2. Location of important geologic features (adapted from Heath 1971 and Dugan 1974) and type and distribution of surficial deposits in the South Fork of the Stillaguamish River basin. Geologically constrained and unconstrained areas of the basin are also delineated.

The South Fork has had a relatively long history of settlement and resource use dating back to the late 1800s. A railroad was built along the length of the valley to access the gold and silver mining towns of Monte Cristo and Silverton. Several small communities and shake mills followed in the wake of the railroad. Numerous stumps in the riparian areas of the main river and the tributaries attest to the large volumes of lumber needed to support these industries.

Splash-damming came into common practice in the late 1800s and early 1900s as a means to transport logs in streams in the absence of roads or railroads in the Pacific Northwest (Sedell and Luchessa 1982). Splash-damming required that all large woody debris in small tributaries be removed to allow the unhindered passage of a flood wave laden with cedar bolts and logs. The flood waves were created by building log dams in tributary channels. There is evidence that splash-damming occurred in the South Fork including the absence of old-growth, riparian conifers and the lack of large organic debris in many of the tributary stream channels.

Present-day land use, administered primarily by the U.S. Forest Service, includes timber harvest, road construction, and concentrated and dispersed recreation.

Methods

Tributary streams were the focus of this study because they account for 90% of the channel length in the basin and approximately 50% of the coho rearing habitat. The remaining habitat is found within the main river. The type and distribution of surficial deposits, including glacial sediments, were identified using a combination of field mapping and aerial photography. Particle sizes in the surface layer of stream substrates were measured (Wolman 1954) and divided into six size categories: boulders (>256 mm), cobbles (64–256 mm), gravels (22–

64 mm), pebbles (2–22 mm), and sands (<2 mm) (Wentworth 1922). Four to eight measurements were obtained in the low-gradient (<4%) portions of each of 18 tributaries. Sampling sites were located at the heads of riffle and bar areas away from large woody debris and boulders. Areas of active channels (wetted area at summer flow) containing gravel and pebble substrates were divided by the entire wetted channel area to estimate proportions of stream areas containing spawnable size substrates.

Particle sizes of the pavement of tributary stream beds were compared with stream power per unit length or SP (Richards 1982). Stream power can be used as an index of a stream's ability to transport sediment. SP (joules per second per metre) is expressed as

$$(1) \quad SP = \rho g Q S_v$$

where ρ is the fluid density, Q is the discharge determined by the U.S.G.S. method for estimating bankfull discharge for ungauged catchments (Bodhaine and Thomas 1964), S_v is the average gradient of channels in the glacial sediments measured from 1 : 24 000 scale topographic maps (map error $\sim 1^\circ$), and g is gravitational acceleration.

Surface areas of channel units were used to estimate the quantity and distribution of rearing habitats available to juvenile salmonids. Identification of channel units followed the procedure of Bisson et al. (1982) except that large channel structures (e.g. boulder, wood, bedrock, etc.) were also inventoried. Channel unit lengths and widths were visually estimated with every tenth unit measured for calibration (Hankin 1984; Hankin and Reeves 1988). Surface areas of preferred spawning substrate for coho salmon (gravels between 13 and 75 mm intermediate diameter) were visually estimated.

We computed the changing rate of erosion and sediment yield of glacial sediment (primarily sand, silt, and clay) from the

South Fork basin and its tributary watersheds over the past 14 000 yr by dividing the volume of sediment eroded between fluvial terraces of known age. This was done by dating terrace formation at two different elevations in the South Fork basin using radiocarbon dating methods (Isotope Laboratory, University of Washington, Seattle, WA). The top of the horizontal lacustrine deposit at an elevation of 550 m at the mouth of the basin at Verlot was assumed to have stopped forming at the time of deglaciation in the general area (~14 000 yr B.P.) (Crandell 1965). The volume of glacial sediment absent between the top of the lacustrine deposit and a lower, dated terrace level (8700 yr B.P.) was estimated by measuring the map area per contour interval for the elevation difference between the two terraces. This volume divided by the time interval (5700 yr) provided an average rate of sediment export. Similarly, the sediment missing between the 8700-yr-old terrace and a radiocarbon-dated, 1700-yr-old terrace adjacent to the main river was measured, and this yielded another average sediment export rate. For these calculations it was assumed that the two lowest dated terraces have gradients similar to that of the present-day river and that terraces of similar heights above the river have approximately the same age.

Results and Discussion

Reach Scale: Morphology and Distribution of Habitats on Young Fluvial Terraces

Numerous tributaries originating from the steeper bedrock portions of the valley traverse the youngest fluvial terrace prior to their confluence with the South Fork (Fig. 3). Radiocarbon dating of wood obtained from a partially buried deciduous tree (sample QL-4298) from within fluvial deposits yielded an age of 1700 yr B.P. (± 40 yr) for the young terrace.

Stream reaches on the young terrace contain preferred summer rearing habitat for coho salmon because of low gradients ($<2\%$) and because of organic debris and boulders that create pools and provide cover. The type and amount of pool habitats for four streams on the young (lower) terrace are shown in Fig. 4.

Seven of the 18 tributaries examined on the young terrace are oriented downvalley on the river valley floor and subparallel to the main river channel. This orientation contributes to the length and low gradient of the tributary streams (Fig. 3). These tributaries include Benson, Gordon, Eldred, Deer, Schweitzer, Hemple, and Heather creeks (Fig. 2). The downvalley orientation of the seven tributaries is a consequence of the surface slope of the young terrace which include a transverse component (towards the river) and a downvalley component. Although the geometry of the terrace explains the majority of the tributaries' downvalley orientation, recent abandoned river channels, overflow channels, and flood levees also appear to contribute. Tributaries that join the South Fork in the absence of the young, 1700-yr-old terrace do not have significant downvalley orientations.

Approximately 70% of summer pool area contained in all the tributary basins is located in tributaries on the young terrace. The tributary pool habitat contained on the young terrace and the pool habitat within the active channel of the South Fork account for 85% of the preferred coho salmon rearing habitat in the watershed, an area that comprises less than 10% of the entire watershed area. Rearing habitats of the young terrace tributaries appear similar to those of the terrace tributaries described by Cederholm and Scarlett (1982) in the Clearwater Basin in Washington State.

Reach Scale: Morphology and Distribution of Habitats on Older Fluvial Terraces

In contrast with low-gradient tributary reaches on the young terrace, upstream reaches flowing through older terraces (>1700 yr old) have steeper gradients (average 4.6%). The steep gradients have developed as a consequence of the Stillaguamish River incising into the glacial sediments following deglaciation (approximately 250 m over 14 000 yr). This results in an increase in elevation difference between the river and its tributaries and a corresponding increase of channel gradients. Differences in channel gradients between younger and older terrace reaches correspond to differences in salmonid habitats as delineated by channel units.

Paired comparisons of the two reach types in Benson, Eldred, Deer, and Schweitzer creeks demonstrated that channels incised into upper, older terraces have a higher proportion of rapids and cascades (Fig. 4). The average percent pool area of 38% in upper terrace reaches compares with 57% for reaches in young terraces. The higher pool percentage in lower gradient channels on the young terrace is caused by the formation of pools in gravel beds at the outside of meanders. The relatively low occurrence of pools formed by large wood in young terrace reaches appears to be related to past logging practices within the riparian zones and removal of large wood from the channels.

Variations in the amounts of woody debris and wood-formed pools in the lower and upper terrace reaches can also be attributed to major disturbances in channels caused by natural processes and by forestry practices. During the last decade, Benson, Gordon, and Blackjack creeks have had very large floods that resulted from the rapid erosion of debris flow dams upstream (Benda and Zhang 1989). These floods altered salmonid habitat by removing large organic debris from within channels. The events in Benson and Blackjack creeks were initiated in logged portions of those tributary watersheds, pointing to the effect of forestry activities on stream habitats.

Low-gradient channels on the young terrace have 46% more channel area in spawning gravels. The abundance of small- to medium-sized gravel in streams on the young terrace is partially attributable to low channel gradient which reduce stream power or sediment transporting competence in those reaches and result in deposition of gravels and pebbles. In contrast, the tributaries flowing through the older, upper-fluvial terraces have steeper gradients and higher stream power, which causes the smaller gravel to be transported through those reaches, leaving behind coarser substrate, such as small boulders. An exception is Schweitzer Creek, which has similar amounts of spawning gravels in both the young and old terrace reaches. The Schweitzer Creek basin is contained almost wholly within sandy glacial deposits (Fig. 2), which reduces the availability of large substrates in channels.

Reach Scale: Variations in Habitat Morphology due to Paleoriver Boulders

Numerous stream reaches on both the young and old terraces contain accumulations of large boulders, 0.5–2 m in diameter, that result in morphological variations in habitats (Fig. 3). These boulders were originally deposited by the Stillaguamish River in a channel that eventually became a terrace. Wood obtained from an older terrace containing the large boulders was dated at 8690 yr B.P. ± 40 yr (QL-4296) and 8750 yr B.P. ± 40 yr (QL-4297), indicating that the Stillaguamish River deposited the boulders during the mid-Holocene Epoch. As the tributary streams eroded through the terrace of the main river,

WATERSHED SCALE

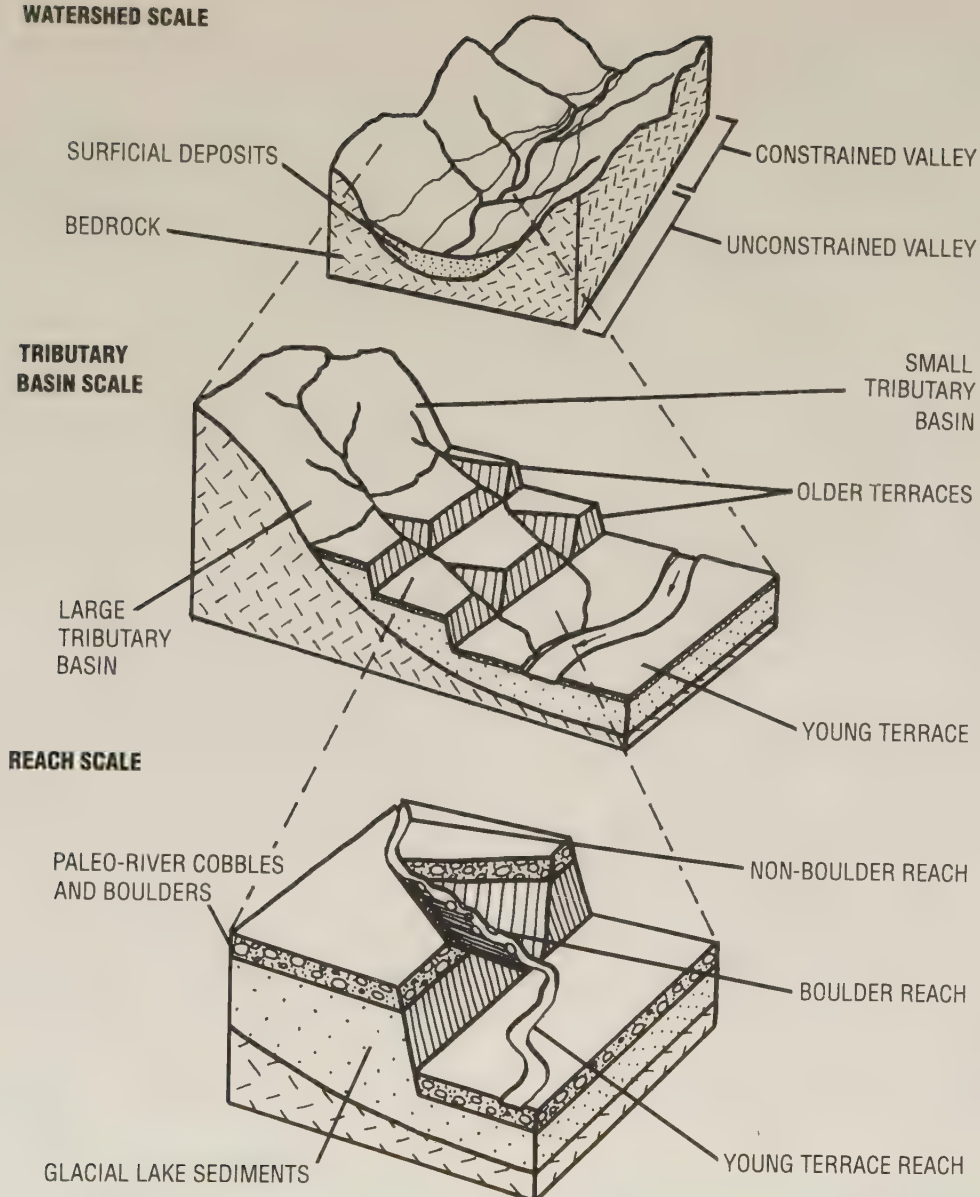


FIG. 3. Schematic diagram showing the three different spatial scales which were used to investigate the relationships between morphology and distribution of salmonid habitats and geomorphology. Important geologic and geomorphic features are indicated.

the large boulders accumulated in tributary channels because they could not be transported by the relatively small discharge of the tributaries.

Stream reaches containing paleoriver boulders are approximately 100 m in length and occur in Turlo, Benson, Wiley, and Schweitzer creeks and several smaller tributaries. Reaches containing large boulders (>0.5 m) are associated with steeper channel gradients. Gradients of seven nonboulder reaches in Schweitzer Creek ranged from 1 to 3% compared with 2.5 to 5% for six boulder reaches. The steep gradients and therefore higher stream powers of boulder-dominated stream reaches result in small areas of spawning gravels. For example, large boulders comprise 28% and gravel (16–64 mm) 21% of the streambed pavement in two boulder-dominated reaches. For nonboulder reaches the opposite trend is evident: only 3% of the streambed is covered with boulders and 50% with gravels (Fig. 5).

Along with woody debris, large boulders are important characteristics in the morphology of pool habitats. Percentage of pools in boulder reaches is 60% compared with 80% in non-

boulder reaches in Schweitzer Creek (Fig. 6). The pools contained in boulder reaches are primarily pocket pools formed in association with boulders and to a lesser extent large wood (Table 1). Pools created by wood comprised 30% of the surface area of the boulder reaches compared with 59% in the non-boulder reaches.

Tributary Basin Scale: Distributions of Spawning Habitat Areas

In the South Fork basin, variation in the amount of spawning gravels between tributary basins is not entirely explained by the presence or absence of the young terrace. The sizes of tributary basins which control stream discharges, and therefore sediment transport competence, influence the relative proportions of spawning gravels between tributaries.

Using the expression for stream power (Richards 1982, eq. 1), we evaluated relationships between discharge, channel gradient, and the proportions of spawning gravels (13–75 mm) in the lower 500-m reaches of 17 tributaries. The analysis indi-

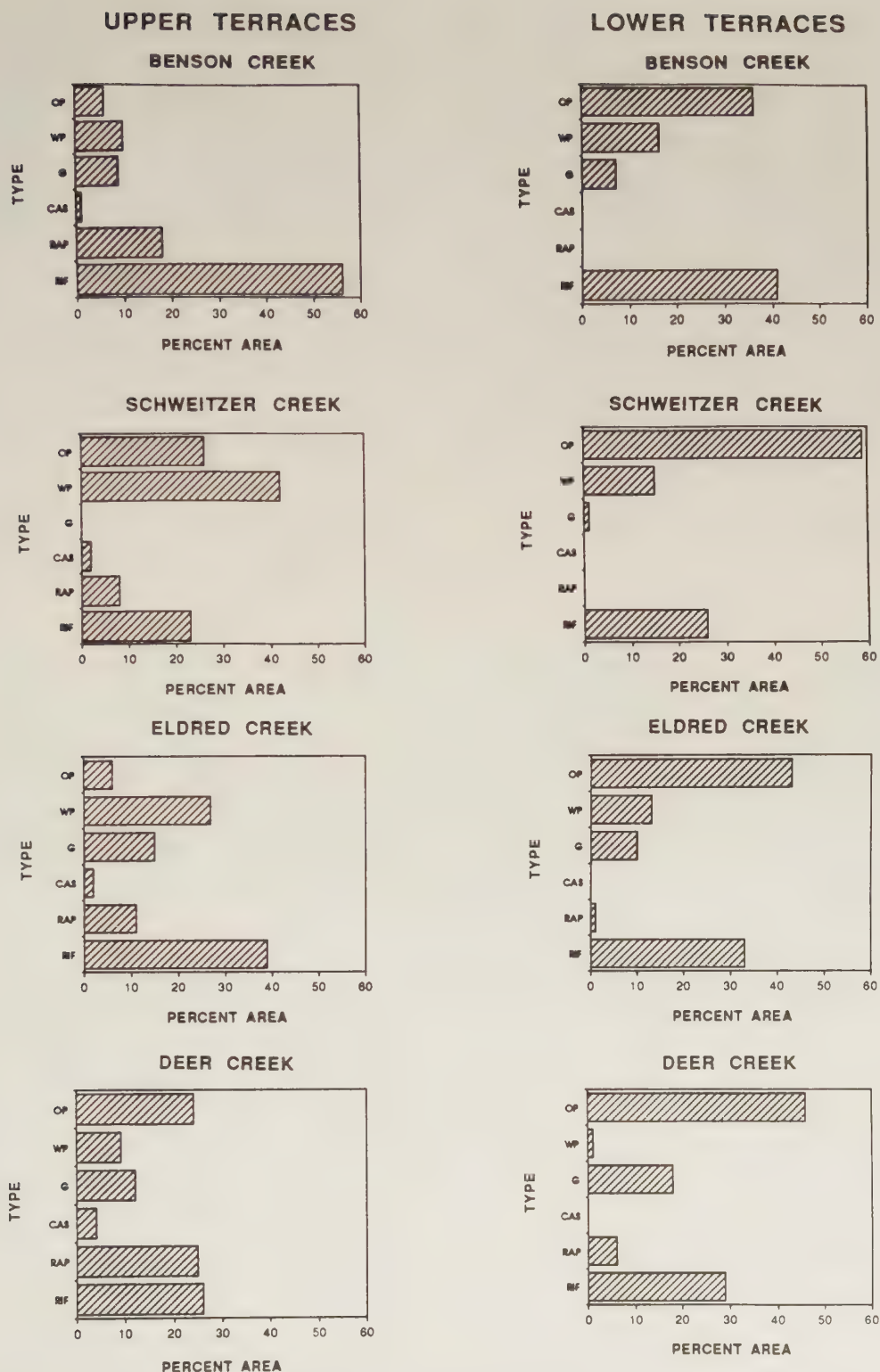


FIG. 4. Distribution of tributary channel units in lower terrace and upper terrace reaches of tributaries expressed as a percentage of total stream surface area. RIF = riffles, RAP = rapids, CAS = cascades, G = glides, WP = pools formed by large organic debris, and OP = all other pool types. Lower terraces are younger surfaces and upper terraces are older surfaces.

cated that tributaries with higher stream powers (large drainage areas and steep channel gradients) are able to transport large cobbles and boulders effectively, although probably at a lower rate than gravel. As a result, these streams have lower proportions of spawning gravels (Fig. 7). In contrast, streams with low stream powers (small basin areas and low channel gra-

dients) are dominated by gravels. In streams with low stream power, large cobbles and boulders are concentrated immediately downstream of alluvial/debris fans (Fig. 2), indicating that selective transport is responsible for the gravel dominance in the lower portions of those tributaries. The boulders contained in the fans are deposited by debris flows and large floods that

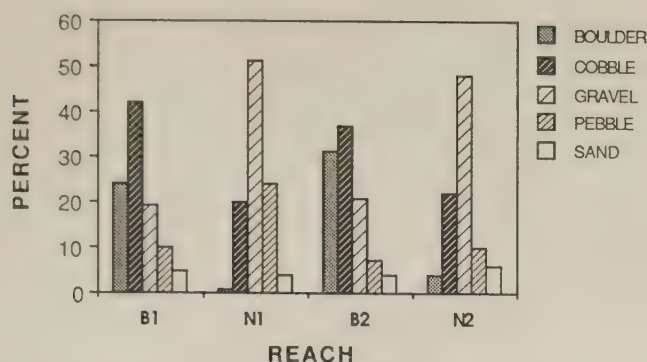


FIG. 5. Particle-size distributions of the pavement layer in boulder and nonboulder reaches of Schweitzer Creek, a tributary to the South Fork of the Stillaguamish River. Sediment sizes include boulders (>256 mm), cobbles (64–256 mm), gravels (16–64 mm), pebbles (1–22 mm), and sands (<1 mm). B1 = boulder reach 1, N1 = nonboulder reach 1, B2 = boulder reach 2, and N2 = nonboulder reach 2.

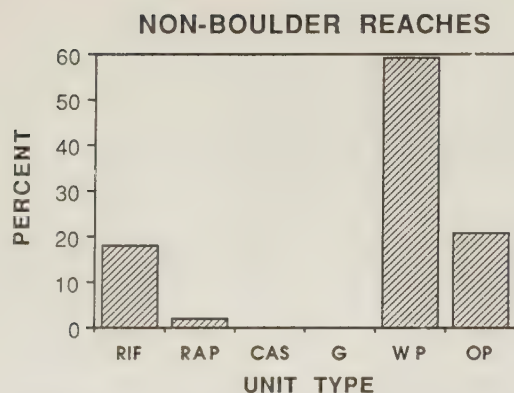
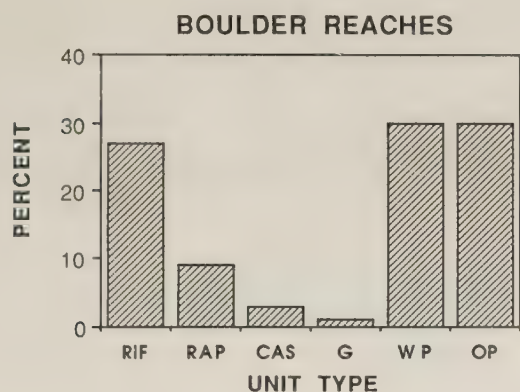


FIG. 6. Distribution of channel units in boulder and nonboulder reaches of Schweitzer Creek, a tributary to the South Fork of the Stillaguamish River. Distributions are expressed as percentages of the total stream surface area in the reach. RIF = riffles, RAP = rapids, CAS = cascades, G = glides, WP = pools formed by large organic debris, and OP = all other pool types.

originate from upper low-order valleys. The majority of tributary basins in the South Fork follow this pattern.

Tributaries basins (<5 km² in area) that have stream reaches oriented downvalley on the young fluvial terrace have the lowest stream powers and the largest spawning gravel areas (e.g. Eldred, Benson, Heather, and Hemple creeks) (Fig. 7). Smaller tributary basins (<3 km²) without stream reaches oriented

TABLE 1. Frequencies of large and medium organic debris (LOD, >5 cm in diameter and >5 m long; MOD, >20 cm in diameter and >3 m long) in boulder and nonboulder reaches of Schweitzer Creek.

	Pools formed by wood (% area)	LOD·100 m ⁻¹	MOD·100 m ⁻¹
Boulder reaches	30	6	17
Nonboulder reaches	59	13	35

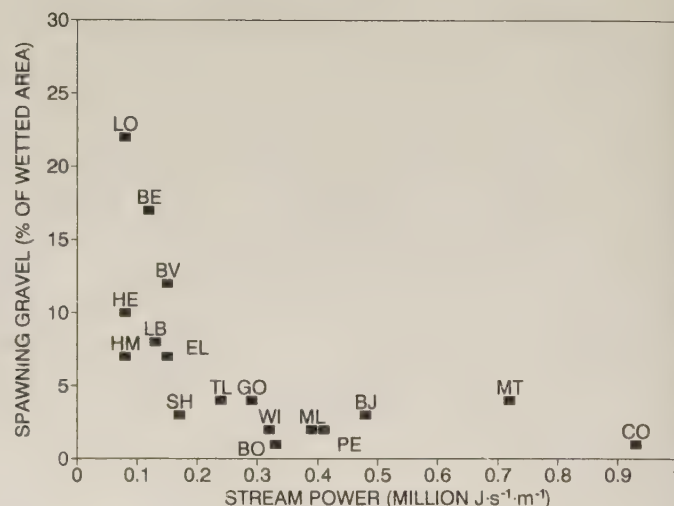


FIG. 7. Relationship of stream power to spawning gravel areas in tributary streams of the South Fork Stillaguamish River. Spawning gravel areas = m²·100 m stream length⁻¹. HE = Heather, HM = Hemple, EL = Eldred, LB = Little Beaver, BE = Benson, LO = Long, BV = Beaver, BO = Boardman, ML = Mallardy, MT = Marten, BJ = Blackjack, CO = Coal, SH = Schweitzer, TL = Turlo, GO = Gordon, W = Wisconsin, and PE = Perry. Stream locations are shown in Fig. 2.

downvalley on the young terrace also exhibit low stream powers and extensive spawning gravel areas (e.g. Long, Beaver, and Little Beaver creeks) (Fig. 7). Tributaries basins with larger areas (>8–10 km²) show the opposite conditions of higher stream power values and lower proportions of spawning gravels. These basins include Boardman, Mallardy, Marten, Blackjack, and Coal creeks.

Other factors also appear to influence the amount and size of substrates in the tributary watersheds. Numerous landslides and debris flows continually occur on hillslopes and in low-order channels of mature forests as well as in clearcut areas in the South Fork. Although debris flows originate in steep, low-order channels, their long travel distance and erosional intensity commonly result in gravel and organic debris deposits in downstream reaches that contain most of the habitats for both anadromous and resident salmonids. These disturbances can cause aggradation and therefore have consequences to salmonid habitats.

Debris flows that occurred during the past decade are most evident in Benson, Wiley, Deer, Gordon, Coal, and Blackjack creeks. The disturbances in Benson, Wiley, and Blackjack creeks originated from within logged portions of the basin, and the remaining events originated from natural forested areas. This raises the question of the effects of timber harvest on frequencies of landslides and debris flows compared with natural conditions. It is important to understand how land use activities are changing the frequency, magnitude, and spatial distribution

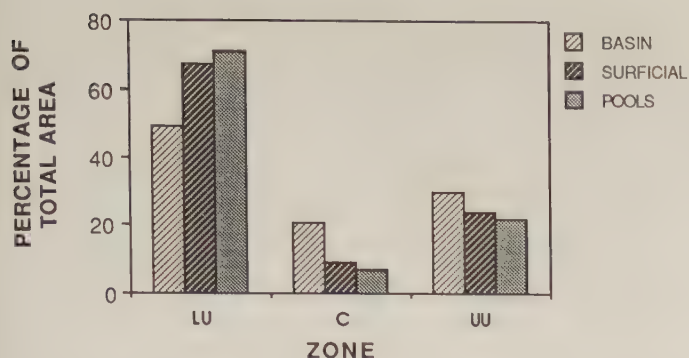


FIG. 8. Percentages of total basin area, total area in surficial deposits, and total summer pool surface area in unconstrained and constrained portions of the South Fork Stillaguamish river basin. LU = lower valley unconstrained area, C = constrained area, and UU = upper valley unconstrained area. BASIN = percent of watershed area, SURFICIAL = percent of total area of surficial deposits, and POOLS = percent of total pool area.

TABLE 2. Summary data for three areas of the South Fork of the Stillaguamish River basin. Locations of areas are shown in Fig. 2.

	Area of river basin		
	Lower unconstrained	Upper constrained	Unconstrained
Number of tributaries	13	4	7
% basin area	49	19	32
% area surficial deposits	69	9	22
% total pools	73	7	20
Mean gradient (%)	5.8	8.8	6.6
Mean % pools	39	27	37

of disturbances across landscapes and how fishes respond or adapt to these disturbances.

Watershed Scale: Distribution of Habitats along the River Valley Floor

A majority of stream channels accessible to anadromous salmonids in the South Fork basin are contained within geologically unconstrained or wide areas of the main river valley. For example, most of the stream surface areas in summer pools (73%) are in the lower unconstrained portion of the valley, an area covering nearly half of the river basin's valley floor. In contrast, the geologically constrained area includes approximately 19% of the basin area and only 7% of the total surface area of summer pool habitat in tributaries (Fig. 8; Table 2).

The wide glacial deposits of unconstrained valley areas favor the development of young terraces and low-gradient tributaries (Fig. 3). For example, six of the seven low-gradient tributaries oriented downvalley on the young terraces are located in the lower unconstrained portion of the river valley floor (Fig. 2). The width of the glacial deposits along the valley ranges from 0.8 to 3.3 km and generally increases downvalley. In the central portion of the valley (Fig. 2), a more geologically constrained area (0.8–2 km wide) lies within the erosion-resistant marbles and cherts of the Chilliwack Group, a lithological unit bounded by two parallel thrust faults.

In summary, the preceding discussions illustrate how analyses of fish habitats in large watersheds can be conducted at several different spatial scales to describe the linkages between the morphology and distribution of habitat and basin geomor-

phology. We expect that many of these geomorphic – fish habitat relationships should also apply to other recently deglaciated basins in the Pacific Northwest. For example, most of the summer pool area should be located in tributaries found on younger terraces of geologically unconstrained or wide river valley floors, and proportionally larger amounts of spawning gravels should be located in the lower portions of smaller tributary basins. These relationships are probably specific to individual physiographic and climatic regimes.

Evolution of Salmonid Habitats from 14 000 yr ago to Present

The recently deglaciated environment of the Pacific Northwest presents an opportunity to develop a hypothesis for the evolution of salmonid stream habitats since continental glaciation (~14 000 B.P.). The hypothesis was based on information on the morphology and distribution of present-day habitats discussed above, our radiocarbon dating and analysis of the erosional history of the watershed, and published data on the evolution of forests in the Pacific Northwest. Our goal was to consider how fish habitats are coupled to geomorphic development of mountain drainage basins following a major continental glacial disturbance.

The present-day South Fork fluvial environment evolved from a highly erosive and sparsely forested landscape that existed immediately following deglaciation (~14 000 yr B.P.). Today, the basin is characterized by relatively low erosion rates, stable channels, and an old-growth conifer forest community (e.g. the Western Hemlock Zone forests, Franklin and Dyrness 1973).

To evaluate how the fluvial environment of the South Fork has changed over 14 000 yr since deglaciation, we estimated erosion rates and sediment yields for different time intervals. Erosion rates and sediment yields in the South Fork were estimated by determining differences in volumes of glacial sediment eroded from fluvial terraces of known age. The age of terrace formation and therefore the magnitude of erosion in the South Fork basin was based on radiocarbon dating. On the basis of this analysis, 90% of the erosion and export of glacial sediment from the South Fork occurred between approximately 14 000 and 8700 yr B.P. This was equivalent to an average sediment yield of approximately $10\,000\text{ tons}\cdot\text{km}^{-2}\cdot\text{yr}^{-1}$. The high rate of sediment yield reflects the immediate lowering of base level of the Stillaguamish River following the removal of the ice dam and the absence of stabilizing vegetation. This sediment yield compares closely with those (postdeglaciation) for several river basins in British Columbia (Church and Ryder 1972).

A modern-day analogue to the fluvial environment of the South Fork during the immediate postdeglaciation period is the North Fork Toutle River in Washington State. The North Fork Toutle River channel currently resides on top of a massive debris avalanche and debris flow deposit that originated from the Mount St. Helens eruption of May 18, 1980. Sediment yield in the North Fork Toutle river during the first several years following the eruption was approximately $35\,000\text{ tons}\cdot\text{km}^{-2}\cdot\text{yr}^{-1}$ and was dominated by fine particles (Janda et al. 1984). The North Fork Toutle River channel, where avulsions are common and banks are unstable, is braided and shallow and partly composed of fine substrates (Lucas 1986).

Between 8700 and 1700 yr B.P., the average sediment yield of the South Fork reduced to approximately $700\text{ tons}\cdot\text{km}^{-2}\cdot\text{yr}^{-1}$. This rate is about 15 times less than the rate during the initial postglacial period. Today, lower sediment

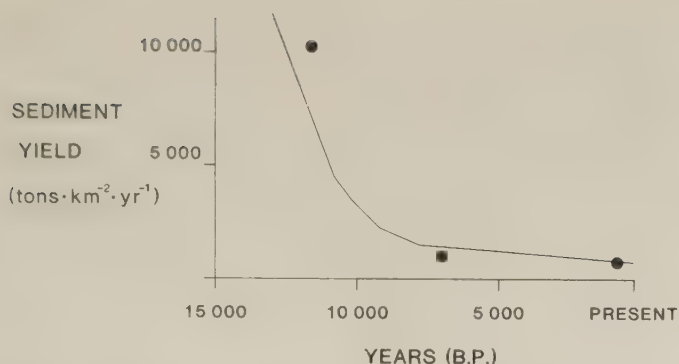


FIG. 9. Average sediment export rates of glacial sediments in the South Fork Stillaguamish River basin since the retreat of the continental ice sheet approximately 14 000 yr ago.

yields for the South Fork basin are assumed to be similar to those of other mountain drainage basins in the Pacific Northwest, that is, approximately $50\text{--}300\text{ tons}\cdot\text{km}^{-2}\cdot\text{yr}^{-1}$ (Swanson et al. 1987).

The comparison of South Fork sediment yields for the three time periods (14 000–8700 yr B.P., 8700–1700 yr B.P., and 1700 yr to present) indicates that erosion decreased dramatically during the initial 5000- to 6000-yr period (Fig. 9). Changes in erosion rates in the basin were also accompanied by changing climatic conditions and forest communities. The erosional history along with changes in climate and forest communities would have strongly influenced the development of salmonid habitats in the watershed.

Changing climatic conditions and forest communities in the South Fork during the later Pleistocene and early to mid-Holocene (14 000–6000 yr B.P.) probably resembled those defined for several locations in Washington State. Past climatic and vegetative patterns have been reconstructed using pollen and macrofossil assemblages for the Cascade foothills at Davis Lake, Washington (Barnosky 1981), the Lake Washington

drainage in the Puget lowland (Leopold et al. 1982), the North Cascade mountain range (Cwynar 1987), and the Olympic Peninsula (Heusser 1974; 1977). Using these data, the probable evolution of climate and forest conditions for the South Fork basin is presented in Fig. 10.

For the Cascade foothills, Barnosky (1981) has shown that a cool climate in conjunction with alpine forests and other conifer vegetation existed from 13 500 to 8000 yr B.P. (Fig. 10). Warmer and drier periods began to appear between 10 000 and 8000 yr B.P. and persisted until 3000–5000 yr B.P. (Fig. 10). Vegetation during the warmer dry interval included *Pseudotsuga* (Douglas fir), *Abies* (spruce), *Alnus rubra* (red alder), and several grass species. The absence of mesic taxa in the pollen assemblages of the early Holocene suggests that vegetation communities in the Cascade foothills of Washington were similar to Douglas fir woodlands and modern oak savanna in the southern Willamette Valley of Oregon (Brubaker 1991).

A shift to a more cool and moist climate began around 7000–5000 yr B.P. in western Washington and continues to persist today. This led to the colonization of more mesic taxa, such as *Tsuga heterophylla* (western hemlock) and *Thuja plicata* (western red cedar) (Fig. 10). An examination of pollen spectra indicates that old-growth Douglas fir forests similar to today's forests did not occur in the foothills of the Cascade mountain range prior to approximately 6000 B.P. (Brubaker 1991).

Therefore, the palynological studies indicate that a subalpine forest community with relatively small trees probably existed in the South Fork basin during the initial several-thousand-year period following deglaciation when erosion rates were high. Although sometime after 8000 yr ago, a cool and moist climate led to increasing amounts of Douglas fir and true fir forests, old-growth Douglas fir forests did not become fully developed until sometime after 6000 yr B.P. when erosion rates had declined significantly. Hence, organic debris of the size that occurs today would not have existed in channels prior to about 6000 yr ago in the South Fork basin.

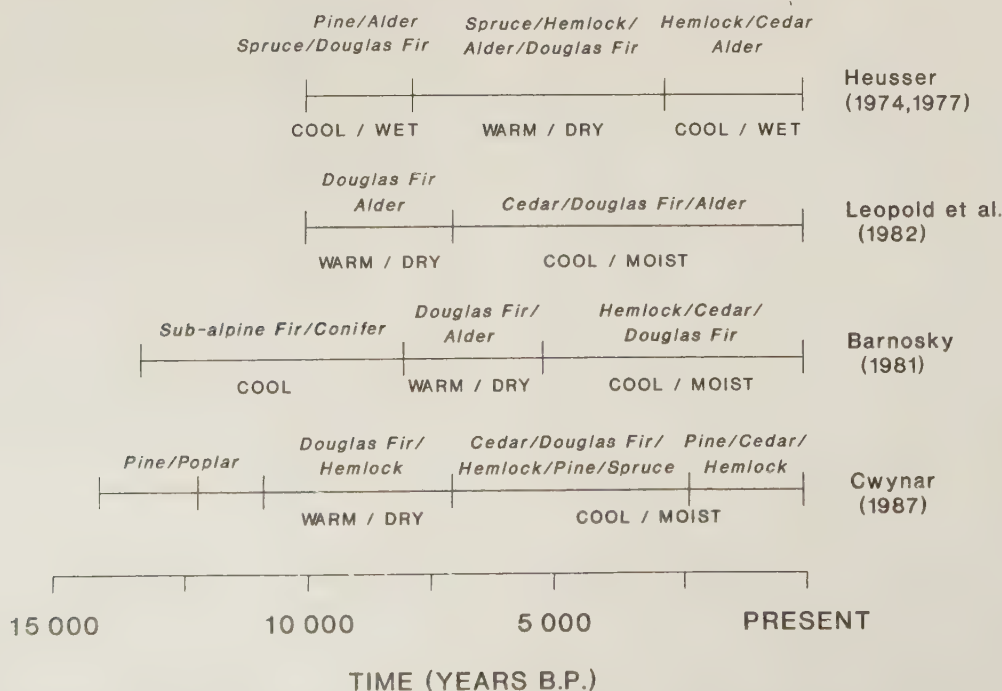


FIG. 10. Polynological studies from the Pacific Northwest showing the changing vegetation patterns and climate for the 14 000-yr period following the retreat of the continental ice sheet.

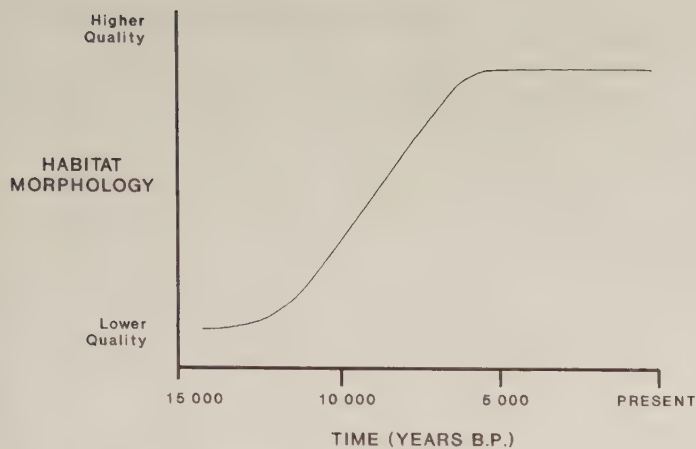


FIG. 11. Change in salmonid habitat morphology over time since glaciation as inferred from present-day morphology and distribution of habitats, the erosional history of the basin, and published literature on the development of forests in the Pacific Northwest.

Fish habitats in low-gradient tributaries in the South Fork immediately following deglaciation were most likely abundant but of poor quality. Habitats were probably characterized by cohesive substrates, shallow and braided channels, turbid waters, and small organic debris. Such sediment-rich and unstable habitats would provide lower quality spawning and rearing habitats for fish.

Between 10 000 and 8700 yr B.P., rapidly declining erosion and sediment yields (Fig. 9), coupled with developing conifer forests during the early to mid-Holocene (Fig. 10), suggest rapidly improving stream habitats. Specifically, the quality and stability of stream gravels and the size of woody debris in channels would have been increasing. Further reduction and stabilization of erosion and sediment yields sometime after 8700 yr B.P. and the establishment of old growth conifer forests in the South Fork basin (~6000 yr B.P.) imply that stream habitats attained their present-day morphology during the mid-Holocene, between approximately 8000 and 6000 yr B.P. (Fig. 11). A seeming paradox, however, is that as habitat quality improved over time since deglaciation, there occurred concomitantly a diminution of low-gradient salmonid spawning and rearing habitat area in the watershed.

The high sediment yields following deglaciation were the result of rapid incision of the South Fork river and its tributaries into the glacial sediments (~250 m in 14 000 yr). As a consequence, the valley floor containing the youngest terrace and therefore the highest quality salmonid habitat has been diminishing in width over time since deglaciation. The width of the valley floor immediately following deglaciation and prior to river incision into glacial deposits was the width of the entire glacial deposit filling the valley which averaged 3300 m at six cross sections (Fig. 12). Hence, low-gradient tributaries containing lesser quality habitat would have existed across the entire valley floor at that time (~14 000 yr B.P.). The width of a lower terrace dated at 8700 yr B.P. (located 30 m above the present-day river) indicates that the valley floor necessary for the development of higher quality habitat diminished in width by approximately 300% in 5000 yr (Fig. 12).

Although stream habitats had probably attained today's morphology between approximately 8000 and 6000 yr ago, the width of the valley floor, and therefore lengths of low-gradient reaches, has since been in decline because of river incision into glacial deposits. Therefore, salmonid habitats in the South Fork basin would have attained their greatest areal extent during the

ELEVATION (m)

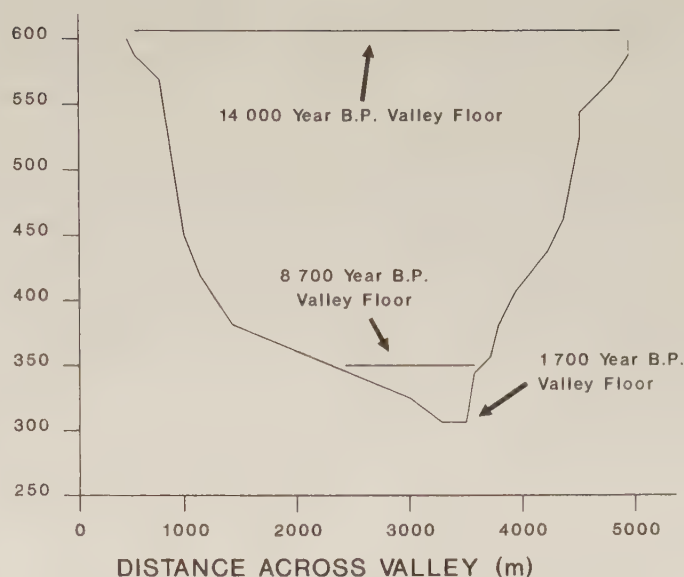


FIG. 12. Changing valley floor widths averaged at six cross sections in the watershed showing the diminution over time of the area containing the low-gradient terrace and salmonid rearing habitat.

mid-Holocene, approximately 7000 yr ago, and they have been in slow, steady decline ever since.

The incision of the South Fork into the glacial sediments caused a replacement of low-gradient streams with higher gradient streams more suitable for steelhead trout and resident trout species. Therefore, we infer that the South Fork basin during the Holocene may have been characterized by a shifting of habitat fortunes from anadromous to resident salmonid species. Our perspective of the postglacial evolution of the landscape in the South Fork Stillaguamish River valley suggests that anadromous stocks may have attained eighteenth or nineteenth century population levels about 8000–6000 yr B.P. Although a waterfall downstream near the mouth of the South Fork basin denied access to salmonids in recent times, it is not known when the falls became exposed following deglaciation.

Conclusion

Relationships between the morphology and distribution of salmonid habitats and geomorphology were evident at several spatial scales ranging from reach, tributary basin, and the watershed. Many of these relationships should apply to other watersheds in the region that have similar histories of continental glaciation.

Our hypothesis on the evolution of salmonid habitats in the study watershed provides an example of how development of habitats is coupled to formation of landscapes following a major glacial disturbance. Habitats increased in quality in concert with increasing watershed stability and evolution of forests but decreased in quantity as landforms changed over 14 000 yr.

This information can also be used for managing fishes and forests in the river basin. For example, forests on the young terrace could be managed for old-growth characteristics and the recruitment of large organic debris to channels because preferred coho salmon habitats are concentrated in such areas. Harvest activities could be consolidated on hillslopes in the geologically constrained areas of the watersheds that have tributaries not suitable for salmonids. Similarly, more attention

could be directed to potential environmental impacts from forestry activities in areas where salmonid habitats are the most abundant and of high quality, such as in the lower unconstrained portions of the watershed.

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Food Limitation and Interspecific Competition in Snail-Dominated Streams¹

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Hill, W. R. 1992. Food limitation and interspecific competition in snail-dominated streams. *Can. J. Fish. Aquat. Sci.* 49: 1257–1267.

Food limitation and exploitative competition were implicated in two experiments on lotic grazers in eastern Tennessee. In a laboratory feeding experiment, grazing snails (*Elimia clavaeformis*) and caddisfly larvae (*Neophylax etnieri*) from upper White Oak Creek (WOC) grew 7 and 2 times faster, respectively, on a diet of high-biomass periphyton than they did on a diet of WOC periphyton. When fed on the high-biomass periphyton, both grazers accumulated disproportionately more neutral lipid, and snails increased their mean condition index 50% (ash-free dry mass per unit wet mass). Growth and condition of grazers fed WOC periphyton in the laboratory were quite similar to growth and condition in situ, indicating that laboratory conditions were realistic. Analysis of gut contents demonstrated considerable dietary overlap between the two taxa regardless of periphyton diet and suggested that quantity rather than quality of periphyton limited grazer growth and condition in WOC. In a natural experiment, periphyton and *Neophylax* from six streams containing *Elimia* were compared with periphyton and *Neophylax* from six streams that lacked *Elimia*. Periphyton biomass, *Neophylax* diapause mass, and *Neophylax* lipid content were substantially greater in streams lacking *Elimia*, implying that the snail created or exacerbated food-limiting conditions for *Neophylax*.

On a étudié la réduction de la nourriture et la compétition d'utilisation dans deux expériences sur les brouteurs lotiques dans l'est du Tennessee. Dans le cadre d'une expérience sur leur alimentation, des gastéropodes brouleurs (*Elimia clavaeformis*) et des larves porte-bois (*Neophylax etnieri*) du cours supérieur du ruisseau White Oak (WOC) ont subi une croissance 7 et 2 fois plus rapide, respectivement, avec un régime alimentaire de périphyton de forte biomasse par rapport à une alimentation en périphyton du WOC. Quand ils se nourrissaient de périphyton de forte biomasse, les deux espèces de brouteurs ont accumulé beaucoup plus de lipides neutres, et ont augmenté de 50 % leur index de condition moyen (masse sèche sans cendre par unité de masse humide). La croissance et la condition des brouteurs nourris en laboratoire avec du périphyton du WOC étaient tout à fait similaires à celles que l'on a observées sur le terrain, ce qui indique que les conditions de laboratoire se rapprochaient des conditions naturelles. L'analyse du contenu du tractus digestif a démontré une grande similitude dans l'alimentation des deux espèces, sans égard au type de périphyton; cela signifierait que c'est la quantité de périphyton, plutôt que la qualité, qui ralentit la croissance et détériore la condition des brouteurs dans le WOC. Dans une expérience en milieu naturel, le périphyton et le *Neophylax* de six cours d'eau contenant des *Elimia* ont été comparés au périphyton et au *Neophylax* de six cours d'eau qui ne renfermaient pas d'*Elimia*. La biomasse de périphyton, la masse de *Neophylax* en diapause et la teneur en lipides des *Neophylax* étaient beaucoup plus grandes dans les cours d'eau dépourvus d'*Elimia*, ce qui semble signifier que les gastéropodes ont provoqué ou aggravé la diminution de nourriture pour le *Neophylax*.

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On first examination, the potential for food limitation in lotic ecosystems appears small. Catastrophic floods significantly reduce the densities of many stream biota (Siegfried and Knight 1977; Power and Stewart 1987; Grimm and Fisher 1989; Feminella and Resh 1990), and lotic predators are both abundant and effective (Peckarsky and Dodson 1980; Crowl and Covich 1990; Power 1990). Large inputs of allochthonous carbon from riparian vegetation may further reduce the potential of food limitation, at least for organisms adapted to harvesting this resource. Recent studies, however, suggest that inadequate supplies of food limit many stream invertebrates (Hart 1987; Hill and Knight 1987; Lamberti et al. 1987; Gee 1988; Feminella and Resh 1990; Hart and Robinson 1990; Richardson 1991).

Stream grazers may be particularly susceptible to food limitation. Many populations appear to escape control by predators or disturbance and achieve densities at which they deplete their food resource (Stewart 1987; Hill and Knight 1987, 1988; Feminella et al. 1989). Intraspecific competition for limited periphyton has been demonstrated for populations of grazing mayflies (Hill and Knight 1987) and caddisflies (Hart 1987; Lamberti et al. 1987; Feminella and Resh 1990). Physical/chemical factors (such as shear stress, riparian shade, and low nutrient concentrations) that frequently constrain lotic primary production probably predispose grazers to food limitation.

Hard-shelled prosobranch snails of the family Pleuroceridae are abundant in many headwater streams of North America, often achieving densities $>500 \cdot \text{m}^{-2}$ (Anderson et al. 1978; Mancini 1978; Hawkins and Furnish 1987; Richardson et al. 1988). Because these grazing snails are relatively large, they can represent $>90\%$ of invertebrate biomass (Newbold et al. 1983; Miller 1985; Hawkins and Furnish 1987). The standing

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biomass of these snails is also large compared with their primary food: the ash-free dry mass of a pleurocerid snail (*Elimia clavaeformis*) in at least one eastern Tennessee stream is >10 times that of periphyton food source (Newbold et al. 1983). Both laboratory and field studies demonstrate that pleurocerids at natural densities maintain periphyton at low biomass and productivity (Mulholland et al. 1983, 1991; Lamberti et al. 1989; Hill et al. 1992). High snail density, depletion of periphyton by snails, and low periphyton productivity strongly suggest the possibility of food limitation.

Other species of grazers cooccur with pleurocerid snails, although their standing biomass is often much smaller than that of the snails. The effect of snails on these taxa should be considerable if there is little partitioning of the periphyton food resource. Hawkins and Furnish (1987) have suggested that the pleurocerid *Juga* is a competitive dominant in Pacific Northwest streams, and Harvey and Hill (1991) showed that the pleurocerid *Elimia clavaeformis* decreased the densities of chironomids in an eastern Tennessee stream. However, it is not clear that the affected species in these two studies were explicitly grazers, and the effect of the snails could have been that of "bulldozing" rather than through resource depression. Evidence of interspecific exploitative competition between stream grazers is rare (but see McAuliffe 1984; Hart 1985). In general, interspecific exploitative competition has not been widely documented in streams.

In this study, I investigated food limitation of cooccurring pleurocerid snails (*Elimia clavaeformis*) and caddisfly larvae (*Neophylax etnieri*) and explored the effects of interspecific competition for food. Food limitation was first investigated in a laboratory feeding experiment in which *Elimia* and *Neophylax* were given periphyton from their stream of origin or high-biomass periphyton from two sites on a eutrophic stream that lacked the grazers. Gut analysis was performed to examine potential food selectivity and to determine if the two grazers partition their periphyton food resource. I then explored the effect of *Elimia* on *Neophylax* in a natural experiment by comparing resource levels and *Neophylax* populations in streams with and without *Elimia*.

Methods

Study Organisms and Streams

Elimia (= *Goniobasis*) *clavaeformis* (family Pleuroceridae) inhabits tributaries of the Tennessee River (Goodrich 1940). Much research has focused on the snail's consumption of periphyton and its resulting ecosystem effects (Elwood and Goldstein 1975; Elwood et al. 1981; Newbold et al. 1983; Mulholland et al. 1983; Hill and Harvey 1990; Steinman et al. 1990; Mulholland et al. 1991). *Elimia clavaeformis* grows slowly and appears to be long-lived in eastern Tennessee streams (W. Hill, unpubl. data). Richardson et al. (1988) estimated that individuals of similarly slow-growing *Elimia* species in Alabama were at least 10 yr old.

Neophylax etnieri (Limnephilidae) is a recently described (Vineyard and Wiggins 1987) species of stone-cased caddisfly that grazes periphyton in small, cool streams in eastern Tennessee. *Neophylax etnieri* appears as an early instar in late autumn and goes through four additional instars before entering prepupal diapause in April–May. Diapause lasts until September–October, when larvae pupate within their diapause cases and metamorphose into flying, reproductive adults. Most, if not all, feeding by caddisflies takes place during the larval stage (Wiggins 1977).

Upper White Oak Creek (WOC) is a second-order stream in the Oak Ridge Reservation (Fig. 1; 35°56'N, 84°18'W). Like many headwater streams in eastern Tennessee, WOC flows through deciduous forest and is extensively shaded in spring and summer when leaves are on streamside trees. Its water characteristically contains relatively low concentrations of nutrients (Hill et al. 1992). *Elimia* dominates invertebrate biomass and production in WOC (Smith 1991), but grazing caddisfly larvae (*Neophylax* and glossosomatids) and mayfly nymphs (baetids and heptageniids) are common in early spring. Grazing snails and caddisflies are rare in East Fork Poplar Creek, which originates at the Y-12 Weapons Plant in Oak Ridge. Nutrient concentrations are high in the stream because of industrial discharges from Y-12 and agricultural/suburban inputs downstream, and canopy cover is negligible at many locations (including our periphyton collection sites). As a consequence of the scarcity of shade, the absence of grazing snails, and the relatively high nutrient concentrations, periphyton biomass is elevated in East Fork Poplar Creek.

Laboratory Feeding Experiment

Elimia clavaeformis and *Neophylax etnieri* from WOC were fed one of three diets in the laboratory: (1) periphyton from upper WOC, (2) high-biomass periphyton from upstream East Fork Poplar Creek (EF1), or (3) high-biomass periphyton from downstream East Fork Poplar Creek (EF2). Eleven miniature streams (16.4 cm in diameter) in which water was circulated at approximately $10 \text{ cm} \cdot \text{s}^{-1}$ by a fine stream of air bubbles served as growth chambers for the grazers (see Mackay 1981 for details of chamber design and function). Both *Elimia* and *Neophylax* are found in a variety of current velocities in WOC, ranging from 0 to $>30 \text{ cm} \cdot \text{s}^{-1}$ (personal observation). Four of the chambers received periphyton-covered rocks from WOC, four received periphyton-covered rocks from EF1, and three received periphyton-covered rocks from EF2. The rocks (approximately 5 cm in diameter) were packed tightly as a single layer on top of a base of sand and gravel in the chambers. The rocks in the chambers were replaced with freshly collected rocks every 2 d to ensure natural algal assemblages and biomass levels. Water (approximately 1 L) from WOC was added to each chamber and was changed daily with freshly collected water from WOC. Chambers were located inside of a large, circular tank and were immersed in temperature-regulated water that flowed through the tank. Water temperature was measured daily and adjusted to match WOC temperature, which ranged from 11.6 to 17.9°C during the study. A 1000-W metal halide lamp provided irradiance of approximately $120 \mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in the chambers.

Twenty snails and six fourth-instar *Neophylax* larvae from WOC were added to each chamber on 20 April 1989. These numbers were equivalent to densities of 1225 snails and 368 larvae $\cdot \text{m}^{-2}$ of chamber bottom area. Snail densities in WOC surveyed just before the beginning of the experiment with 0.05- m^2 quadrats were 1544 ± 139 (mean $\pm$ SE, $n = 10$) snails $\cdot \text{m}^{-2}$. Density of fourth-instar *Neophylax* larvae was not surveyed at this time, but 300–400 late-instar larvae $\cdot \text{m}^{-2}$ is not uncommon in WOC (personal observation). Snails added to the chambers were the modal size in WOC (approximately 100 mg blotted wet mass and 4.6 mm shell width). The 20 snails were weighed as a group before being added to each chamber. Initial ash-free dry mass (AFDM) of *Neophylax* was estimated with a subsample from the batch of larvae that were added to the chambers; initial AFDM was $0.43 \pm 0.01 \text{ mg}$ ($n = 14$). During the experiment, individual *Neophylax* were collected within



FIG. 1. Oak Ridge Reservation streams and collecting sites used in this study. WOC = upper White Oak Creek, EF1 = upper East Fork Poplar Creek, EF2 = downstream East Fork Poplar Creek, ORNL = Oak Ridge National Laboratory, Y-12 = Y-12 Weapons Plant. Filled circles denote streams containing *Elimia*; open circles denote streams lacking *Elimia*. 1 = First Creek, 2 = Gum Hollow Creek, 3 = Ish Creek, 4 = Mill Branch, 5 = Walker Branch, 6 = upper White Oak Creek, 7 = Bear Creek Tributary, 8 = Fifth Creek, 9 = Melton Branch, 10 = Northwest Tributary, 11 = Pinhook Creek, 12 = UT Farm Stream.

2 d of the onset of diapause and were frozen for later analysis of AFDM and lipid content.

The experiment ended when the last *Neophylax* began diapause on 27 May. At the end of the experiment, snails from each container were blotted dry, weighed, and then frozen for subsequent analysis of AFDM and lipid content. Four snails from each chamber were later thawed, measured for width, and dissected from their shells. All of the *Neophylax* larvae were also thawed and removed from their cases. Individual snails and *Neophylax* were then dried to constant mass at 60°C and weighed. Neutral lipids were extracted from dried animals with 5 mL of anhydrous ethyl ether in individual scintillation vials for 2 d, after which the remaining tissue was weighed (Dobush et al. 1985). The tissue was then ashed at 480°C and weighed again. Lipid mass was calculated as mass loss after ether extraction; AFDM was calculated as unextracted dry mass minus ash mass. Average growth rate of the snails in each chamber was calculated as the difference in average blotted wet mass between the beginning and end of the experiment divided by 38 d. Growth rates of individual *Neophylax* larvae were calculated as the difference between final AFDM and initial AFDM divided by the number of days before the onset of diapause. A condition index for snails similar to that of bivalves (Baird 1958) was calculated as AFDM divided by estimated blotted wet mass. Blotted wet mass was estimated from measured width and non-linear regression of wet mass versus width ($r^2 > 0.90$).

To determine if the growth of *Elimia* and *Neophylax* was significantly affected by chamber conditions, growth and condition parameters were measured on unconstrained animals in

WOC. *Elimia* growth in situ in WOC was monitored by glueing individually numbered yellow bee tags to 99 snails, weighing (blotted wet mass) the snails coincident with the start of the laboratory experiment, and then reweighing tagged snails collected (45 of the original 99) from WOC at the end of the laboratory experiment. Lipid and condition index were also determined from a sample of snails taken from WOC at this time. Diapausing *Neophylax* larvae were collected from WOC at the end of the laboratory experiment. Lipid and AFDM were determined for these larvae as described above.

Gut Analysis

Resource overlap and selective ingestion were investigated through microscopic examination of grazer gut contents and periphyton. Snails (modal size) and fifth-instar *Neophylax* larvae were collected from WOC and allowed to graze on one of the three periphyton types in growth chambers. After 24 h, snails and *Neophylax* were retrieved from the chambers and frozen. Four snails and four *Neophylax* from each periphyton type were subsequently thawed and dissected: stomach contents of snails were removed with forceps and micropipet, while the entire front half of the digestive tract of *Neophylax* was removed with forceps. Gut contents were placed on glass slides, and 150 algal cells and filaments from each animal were counted at 400×. Periphyton samples were brushed from three rocks of each periphyton type; algal cells were preserved in Lugol's solution and later examined with an inverted microscope: 150 cells or filaments were counted at 400×. Algal cell counts for both gut contents and periphyton were converted to biovolume

by multiplying counts by estimated biovolume per cell. Biovolume per cell for each alga taxon was estimated by measuring the dimensions of 10 cells of each taxon and using the average of these measurements in geometric formulae of representative shapes.

Differences in gut fullness were estimated in two to four individuals that grazed each periphyton type for 24 h (above). Entire digestive tracts of *Neophylax* were weighed immediately after dissection. Because snail digestive tracts were difficult to excise cleanly, the quantity of periphyton ingested by snails was approximated by extracting macerated individuals in 5 mL of dimethyl sulfoxide (DMSO) overnight and analyzing the extract spectrophotometrically at 664 nm, the chlorophyll *a* absorption peak.

Periphyton Analysis

The amount of periphyton on the rocks put into the chambers was estimated four times during the growth experiment. Four rocks were randomly selected at these times from rocks freshly collected from each site. Periphyton chlorophyll *a* on these rocks was determined by overnight extraction in DMSO and spectrophotometric analysis of the extract (Boston and Hill 1991). Periphyton AFDM was quantified once at the end of the experiment by brushing and rinsing the upper surface of rocks into aluminum pans, drying the periphyton suspension to constant mass at 60°C, weighing, ashing at 480°C, and reweighing.

Stream Survey

The impact of *Elimia* on *Neophylax* was explored by sampling diapausing populations of *Neophylax* in 12 headwater streams on the Oak Ridge Reservation, six of which lacked *Elimia* (Fig. 1). Ten diapausing *Neophylax* larvae were collected in a random manner from riffle-run sections of each stream in April and May 1990; larvae were frozen for later analysis of AFDM and lipid content.

Periphyton and snail densities in the survey streams were sampled on 21 September 1990. A 5.31-cm² neoprene sampling ring (Hill and Knight 1987) was used to sample three separate rocks taken from riffle-run areas in each stream: the ring was pressed against each rock, partially filled with distilled water, and the periphyton inside the ring was dislodged with a stiff brush. The resulting periphyton slurry was collected with a syringe and analyzed for AFDM. Snail densities in streams that contained *Elimia* were estimated by counting the number of snails within three 0.03-m² quadrats in riffle-run areas in each stream.

Water chemistry parameters in the streams with and without snails were compared on two dates: alkalinity and pH were measured from samples taken 10 October 1990 and nitrate and soluble reactive phosphorus (SRP) were analyzed from samples taken 11 December 1990. Nitrate and SRP were analyzed with a Lachat automatic analyzer using Environmental Protection Agency (1979) methods.

Data Analysis

Analysis of variance (ANOVA) as performed by PROC GLM (SAS Institute 1985) was used for all hypothesis testing. Data were logarithmically transformed when variances were associated with means in order to reduce heteroscedasticity. Chlorophyll *a* data from the growth experiment were analyzed in a two-way ANOVA, with time as one factor and periphyton source as the other. A complete randomized design was used

to analyze grazer growth and condition data in the laboratory growth experiment; individual chambers served as experimental units. Values from individual animals served as experimental units in the analysis of gut mass and ingested pigments. Individual streams served as experimental units in the analysis of differences between streams in the stream survey experiment.

Results

Laboratory Feeding Experiment

Periphyton biomass and algal composition

Both EF1 and EF2 diets provided significantly more algal biomass for grazers than did the WOC diet throughout the laboratory feeding experiment (Fig. 2). AFDM measurements made at the end of the experiment also documented the low biomass of WOC periphyton: WOC AFDM was 0.12 ± 0.001 mg·cm⁻² ($n = 3$), while EF1 AFDM was 1.62 ± 0.24 mg·cm⁻² ($n = 3$) and EF2 AFDM was 3.08 ± 0.65 mg·cm⁻² ($n = 3$). Although mean AFDM and chlorophyll *a* of EF2 periphyton were greater than those of EF1 periphyton, the difference was not statistically significant (Fisher's protected least significant difference, $\alpha = 0.05$). The standing biomass of *Elimia* in WOC, estimated by multiplying mean density by the average AFDM of modal-size snails, was 1.05 mg·cm⁻², nearly 10 times the standing biomass of periphyton.

The community structure of WOC periphyton was very simple, consisting mostly of *Stigeoclonium* sp. prostrate cells and short filaments of *Phormidium tenue* (Table 1). *Stigeoclonium* and *Phormidium* also dominated EF1 periphyton (Table 1). Periphyton from EF2 was three-dimensional: coarse filaments of *Cladophora* sp. formed a framework for diatom accumulation above a basal layer of *Stigeoclonium*. *Cladophora* filaments were very large (up to 200 μ m in diameter and many centimetres in length) and were not found in the guts of snails and *Neophylax*, so they were not included with the microalgae in Table 1.

Snail responses to periphyton diets

Elimia growth on WOC periphyton was clearly food limited. Snails that grazed the high-biomass periphyton from EF1 and EF2 accumulated wet mass 5 times faster than snails that grazed WOC periphyton (Fig. 3A; Table 2) and had significantly larger condition indices (Fig. 3B; Table 2). If tissue growth is calculated as wet growth $\times$ condition index, then snails grazing EF1 and EF2 periphyton accumulated AFDM 7 times faster than snails that grazed WOC periphyton (Fig. 3C; Table 2). Storage lipid was disproportionately lower in WOC-fed snails than in snails fed high-biomass periphyton (Fig. 3D; Table 2). ANOVA of wet mass growth, condition index, AFDM growth, and lipid content confirmed highly significant effects of diet, due primarily to low WOC values (Table 2). Chamber effects appeared negligible, as growth, condition index, and lipid content of snails grazing WOC periphyton in the chambers were very similar to in situ values. Because *Elimia* growth is strongly size specific (W. Hill, unpubl. data), in situ growth rates were calculated only for tagged snails 90–120 mg initial wet mass ($n = 15$) that were recovered from WOC. This range corresponded to the range of initial wet masses of snails in the growth chambers.

Neophylax responses to periphyton diets

Neophylax growth on WOC periphyton was also food limited. Larvae that grazed EF1 and EF2 periphyton took less time to reach diapause (Fig. 4A; Table 2) and were 50–75% larger than larvae that grazed WOC periphyton (Fig. 4B; Table 2).

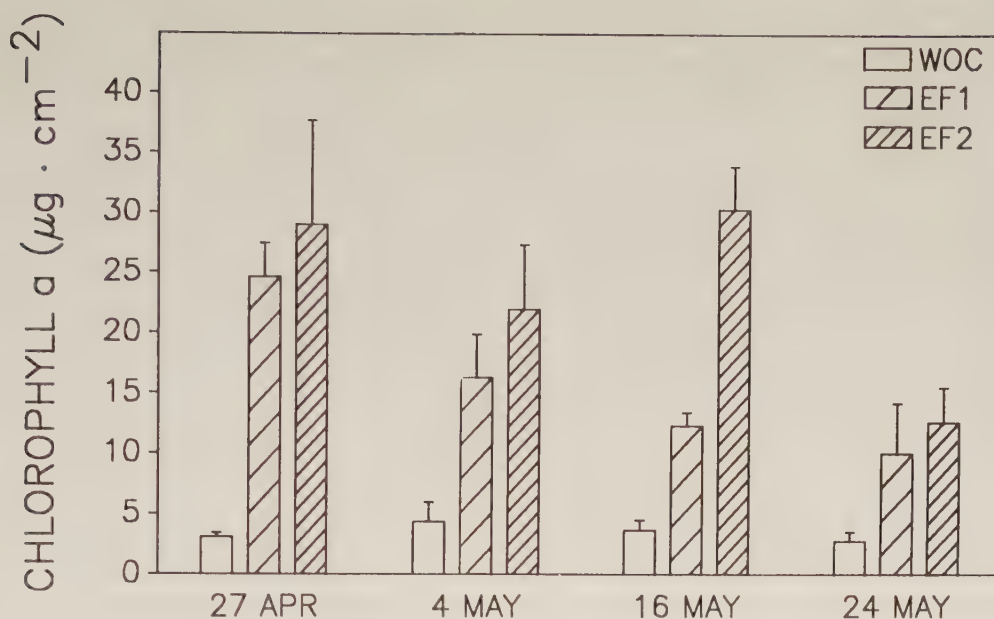


FIG. 2. Chlorophyll *a* on rocks used in the laboratory feeding experiment. Means $\pm$ 1 SE of four rocks for each date and collecting site are shown. Analysis of site differences: $F_{2,33} = 107.3$, $P < 0.001$; WOC < EF1 = EF2 (Fisher's protected least significant difference, $\alpha = 5\%$).

TABLE 1. Microalgae in periphyton and grazer guts. Numbers in parentheses indicate means $\pm$ SE of relative biovolume; only taxa contributing >2% of total biovolume are listed.

Periphyton origin	Periphyton	Elimia gut	Neophylax gut
WOC	<i>Stigeoclonium</i> sp. (81.9 $\pm$ 7.4) <i>Phormidium tenue</i> (15.0 $\pm$ 7.4)	<i>Stigeoclonium</i> sp. (93.4 $\pm$ 1.8) <i>Phormidium tenue</i> (4.8 $\pm$ 1.6)	<i>Stigeoclonium</i> sp. (88.2 $\pm$ 7.1) <i>Phormidium tenue</i> (9.2 $\pm$ 5.6)
EF1	<i>Stigeoclonium</i> sp. (45.6 $\pm$ 19.0) <i>Phormidium tenue</i> (33.2 $\pm$ 20.1) Unicellular chlorophyte (13.8 $\pm$ 4.7) <i>Navicula</i> sp. (5.8 $\pm$ 2.0)	<i>Stigeoclonium</i> sp. (39.4 $\pm$ 19.4) Unicellular chlorophyte (37.4 $\pm$ 13.5) <i>Surirella</i> sp. (8.3 $\pm$ 8.3) <i>Achnanthes</i> sp. (6.8 $\pm$ 3.4) <i>Phormidium tenue</i> (4.2 $\pm$ 4.2) <i>Gomphonema</i> sp. (2.3 $\pm$ 2.3)	<i>Stigeoclonium</i> sp. (37.2 $\pm$ 15.6) <i>Phormidium tenue</i> (35.4 $\pm$ 12.9) Unicellular chlorophyte (19.8 $\pm$ 19.8) <i>Navicula</i> (6.0 $\pm$ 3.4)
EF2	<i>Rhoicosphenia curvata</i> (26.9 $\pm$ 9.8) <i>Stigeoclonium</i> sp. (24.0 $\pm$ 7.9) <i>Melosira varians</i> (20.5 $\pm$ 14.3) <i>Surirella</i> sp. (17.7 $\pm$ 8.8) <i>Gomphonema</i> sp. (5.7 $\pm$ 0.9)	<i>Rhoicosphenia curvata</i> (59.8 $\pm$ 17.7) <i>Gomphonema</i> sp. (20.8 $\pm$ 10.6) <i>Cocconeis pediculus</i> sp. (4.5 $\pm$ 5.9) <i>Cocconeis placentula</i> sp. (4.5 $\pm$ 1.2) <i>Melosira varians</i> (3.9 $\pm$ 3.9)	<i>Rhoicosphenia curvata</i> (47.1 $\pm$ 16.0) <i>Stigeoclonium</i> sp. (40.8 $\pm$ 18.7) <i>Cocconeis placentula</i> sp. (3.8 $\pm$ 2.8) <i>Gomphonema</i> sp. (3.4 $\pm$ 2.1)

Larval growth (final mass — estimated initial mass)/number of days before diapause) during the experiment was two times greater for larvae grazing EF1 or EF2 periphyton than for larvae grazing WOC periphyton (Fig. 4C; Table 2). EF2 periphyton produced the highest percentage of storage lipid, while WOC periphyton produced the lowest (Fig. 4D; Table 2). Chamber effects were minimal for *Neophylax*: larvae fed WOC periphyton in the chambers diapaused at approximately the same time, with the same mass, and with approximately the same lipid content as larvae in situ.

Gut Contents

Considerable resource overlap was evident in the similarity between the gut contents of snails and *Neophylax* for each periphyton diet (Table 1). The algae in the gut contents of both grazers was generally representative of what was available in their periphyton diet, although *Cladophora* filaments in EF2 periphyton were avoided. Prostrate cells and short filaments of *Stigeoclonium* dominated the guts of snails and *Neophylax* that grazed WOC periphyton. *Stigeoclonium* was the most common

component of the gut contents of grazers that fed on EF1 periphyton, but *Phormidium tenue*, unicellular chlorophytes, and small diatoms were also present. Diatoms (especially *Rhoicosphenia curvata*) dominated the guts of snails and *Neophylax* fed EF2 periphyton; no *Cladophora* filaments were found in the guts of either grazer.

Microscopic examination revealed that the guts of snails and *Neophylax* that fed on WOC periphyton contained relatively small quantities of microalgae. This was confirmed by the greater amount of pigments absorbing at 664 nm (presumably chlorophyll *a* and its breakdown products) in snails that grazed EF1 and EF2 periphyton and by the greater gut mass in *Neophylax* larvae that grazed EF1 and EF2 periphyton (Table 3).

Stream Survey

The streams sampled for diapausing *Neophylax* larvae flowed through hardwood forests within a 17-km radius of Oak Ridge National Laboratory (ORNL, Fig. 1). Physical/chemical parameters (temperature, pH, alkalinity, nitrate, SRP) did not

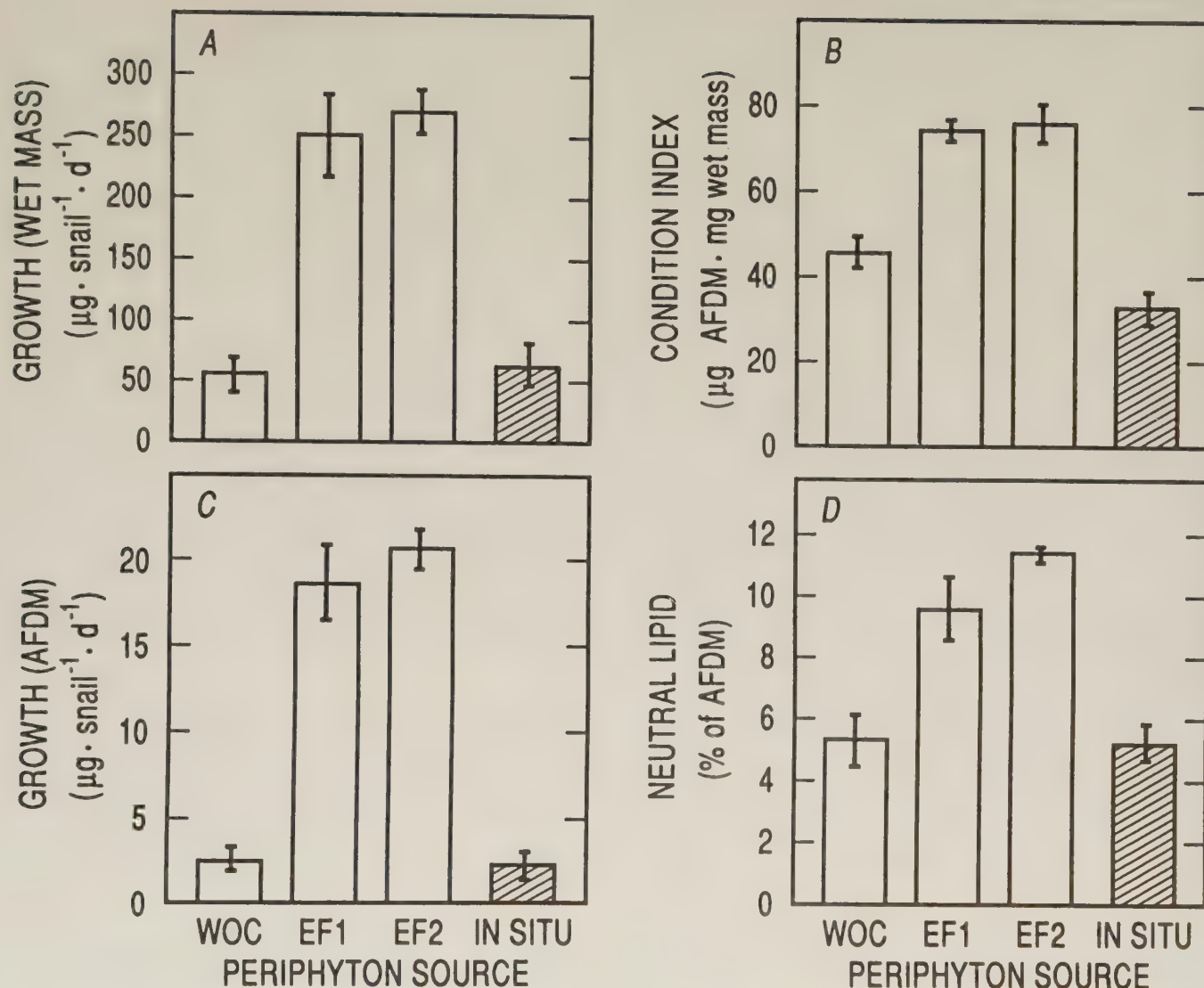


FIG. 3. Effect of periphyton diet on *Elimia clavaeformis*. Bars for WOC, EF1, and EF2 illustrate the means ± 1 SE of individual chamber means. In situ bars illustrate the means ± 1 SE of individual snails that grew freely in White Oak Creek ($n = 15$ for growth and $n = 9$ for condition index and lipid content). Abbreviations for periphyton source follow Fig. 1. AFDM = ash-free dry mass.

TABLE 2. F values and multiple comparisons from ANOVAs of grazer responses to different periphyton diets. PLSD = Fisher's protected least significant difference, $\alpha = 0.05$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Parameter	Transformation	$F_{2,8}$	PLSD
<i>Elimia</i> growth (wetmass)	None	23.84***	WOC < EF1 = EF2
<i>Elimia</i> condition index	None	26.62***	WOC < EF1 = EF2
<i>Elimia</i> growth (AFDM)	None	40.11***	WOC < EF1 = EF2
<i>Elimia</i> lipid (% of AFDM)	None	12.48**	WOC < EF1 = EF2
<i>Neophylax</i> growth period	None	5.75***	WOC = EF1 < EF2
<i>Neophylax</i> AFDM	Log	5.64*	WOC < EF1 = EF2
<i>Neophylax</i> growth (AFDM)	Log	29.92**	WOC < EF1 = EF2
<i>Neophylax</i> lipid (% of AFDM)	None	31.43***	WOC < EF1 = EF2

differ significantly between streams with and without *Elimia* (ANOVA, $P > 0.05$ for all parameter comparisons). Mean *Elimia* density was $643 \pm 129 \cdot \text{m}^{-2}$ in the six streams where the snail was present.

Neophylax larvae from streams lacking *Elimia* were larger and had disproportionately more storage lipid than larvae from

streams containing *Elimia* (Fig. 5A). Formal ANOVA of *Neophylax* AFDM and lipid percentages confirmed that highly significant differences existed between populations from streams with and without *Elimia* (AFDM: $F = 27.13$, $P < 0.001$; % lipid: $F = 55.83$, $P < 0.001$). Except for differences in size, *Neophylax* larvae from all of the streams were indistin-

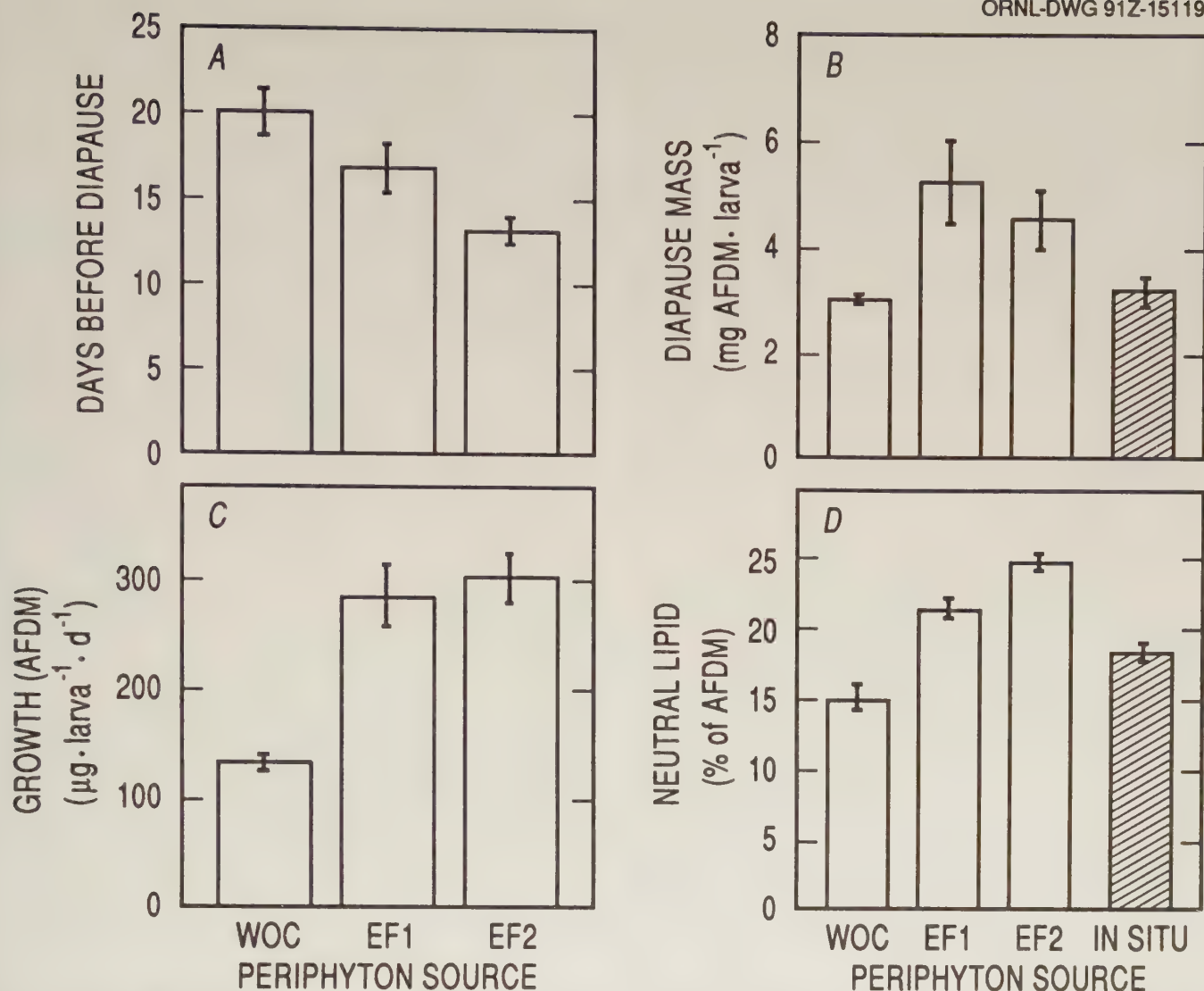


FIG. 4. Effect of periphyton diet on *Neophylax etnieri*. Bars for WOC, EF1, and EF2 illustrate the means ± 1 SE of individual chamber means. In situ bars illustrate the means ± 1 SE of individual larvae that grew freely in White Oak Creek ($n = 25$). Abbreviations for periphyton source follow Fig. 1. AFDM = ash-free dry mass.

TABLE 3. Quantitative analysis (means ± 1 SE) of grazer gut contents. * $P < 0.05$.

Parameter	Periphyton diet			<i>F</i> (df)
	WOC	EF1	EF2	
Algal pigments in <i>Elimia</i> gut (absorbance units ^a ·snail ⁻¹)	0.077 $\pm$ 0.024	0.141 $\pm$ 0.012	0.177 $\pm$ 0.011	9.30* (2,6)
<i>Neophylax</i> gut wet mass (mg·larva ⁻¹)	1.18 $\pm$ 0.17	2.58 $\pm$ 0.92	3.93 $\pm$ 0.25	9.07* (2,4)

^a5-mL DMSO extracts of single snails, quantified in a 1-cm spectrophotometer cell at 664 nm.

guishable from WOC larvae that were reared to adults and identified as *Neophylax etnieri*.

Periphyton biomass was conspicuously higher in streams lacking snails during the spring collection of *Neophylax* larvae. When quantitative samples were taken in the fall of 1990, mean periphyton AFDM in streams that did not contain *Elimia* was 3 times greater than that in streams containing *Elimia* (Fig. 5B). The difference in periphyton AFDM between streams with and without snails was statistically significant ($F = 9.81$, $P < 0.05$, log-transformed data).

Discussion

Elimia and *Neophylax* appeared to be strongly food limited in WOC, as growth and condition of both grazers were substantially increased by periphyton with greater biomass (EF1 and EF2). Close concordance between grazer growth and condition on WOC periphyton in the laboratory and in WOC indicated that laboratory conditions effectively simulated in situ conditions. Grazers in the laboratory and in situ were exposed to similar temperatures, water velocities, water quality, and

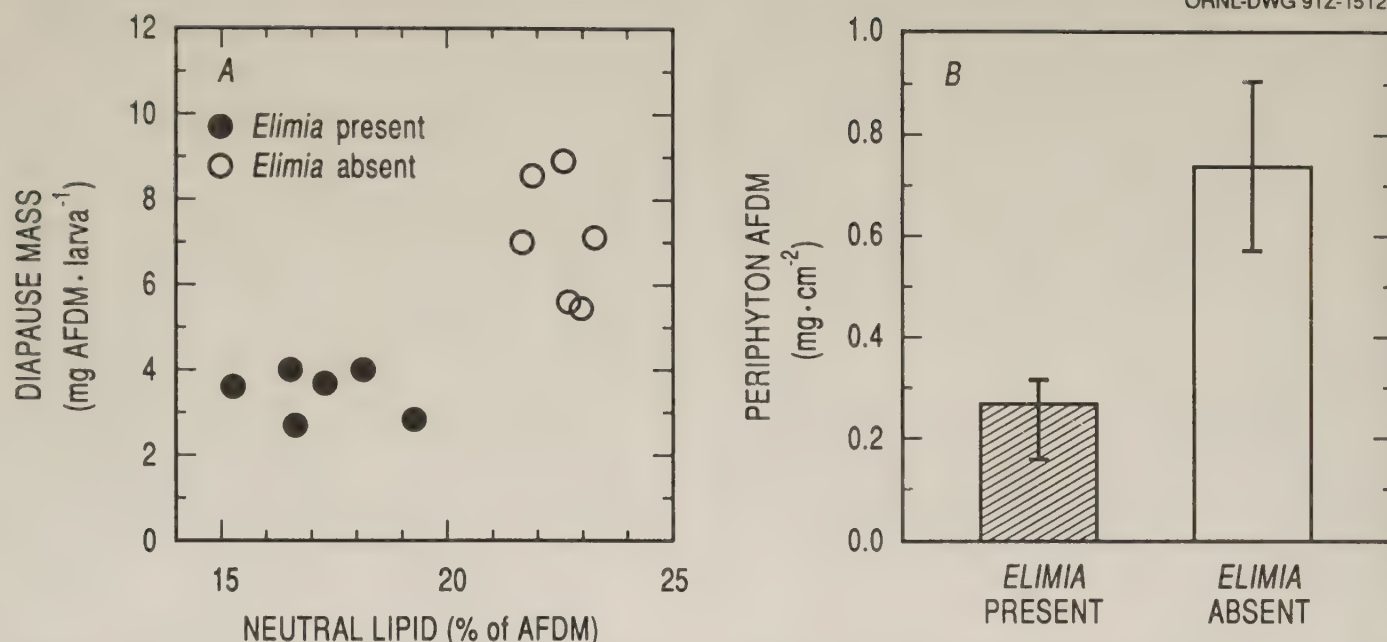


FIG. 5. (A) Average mass and lipid percent in diapausing *Neophylax* larvae in streams with and without *Elimia*, spring 1990. Each point represents the mean value of 10 larvae from one stream. (B) Periphyton biomass in streams with and without *Elimia*, fall 1990. Means $\pm$ 1 SE of six streams with *Elimia* and six streams without *Elimia* are shown.

densities of conspecifics. By replacing the periphyton every other day and providing realistic light and nutrient levels, I ensured that the composition and biomass of WOC periphyton available to grazers in the laboratory strongly resembled that available to grazers in WOC. Biomass and productivity of WOC periphyton were not significantly altered by laboratory conditions (W. Hill, unpubl. data). Although laboratory streams obviously differ from real streams in many respects, concordance between grazer performance in the laboratory (WOC diet) and in situ implies that factors not included in the laboratory (predators, alternative food sources, spates, etc.) did not importantly influence growth in WOC.

It seems likely that grazer growth and condition in WOC were limited by periphyton quantity rather than quality. Periphyton productivity is quite low in WOC (Boston and Hill 1991), and the ratio of grazer biomass to periphyton biomass was $>10:1$ during this study. In a nutrient enrichment experiment in WOC, increased periphyton production appeared to be channelled directly into increased snail density (Hill et al. 1992). In this study, *Elimia* and *Neophylax* that fed on high-biomass periphyton from EF1 and EF2 contained larger quantities of algae in their guts than did *Elimia* and *Neophylax* that grazed WOC periphyton, implying that only submaximal rates of ingestion were possible on the sparse WOC periphyton. If grazers can compensate for low periphyton quality by increasing ingestion rates, periphyton quality may rarely limit their growth.

The snail condition index ([tissue AFDM]/[wet mass]) provided a static measure of food limitation that agreed well with growth. Condition indices have been used extensively to study stress in bivalves (Baird 1958; Bayne et al. 1976; Lawrence and Scott 1982) but to my knowledge have not been applied to gastropods. The snail condition index introduced in this paper is very similar to Baird's (1958) condition index for bivalves ([tissue AFDM]/[internal shell volume]). Wet mass should be very closely related to internal shell volume, as long as shell

thickness and density remain nearly constant. The significant increase in the condition index of snails supplied with extra periphyton biomass indicated that WOC snails were in poor condition and accumulated tissue faster than shell volume when their diet was improved. At some level of food availability, shell growth may constrain tissue growth (e.g. Palmer 1981), but this was clearly not the case for snails grazing WOC periphyton. Changes in the ratio of tissue mass to shell mass in freshwater gastropods have been reported to result from "degrowth" (the absorption of tissue proteins) during winter ice-cover in northern lakes (Russell-Hunter et al. 1984). Our results suggest that condition index may be a useful indicator of the nutritional/physiological state of freshwater snails. Snail growth measured by changes in wet mass alone may bear little relation to tissue growth if (AFDM)/(wet mass) ratios change dramatically during the growth period.

Lipid accumulation is important for both snails and caddisflies. Gastropods store ingested carbon as both glycogen and neutral lipids (Chatterjee and Ghose 1973), but prosobranch metabolism may be "lipid oriented" in that lipids are preferentially utilized during starvation and for reproductive products (Stickle and Duerr 1970; Stickle 1975). Stored lipids obviously benefit *Elimia* in times of food scarcity, such as periods following periphyton-scouring spates. Energy storage is even more critical to *Neophylax* and other insects that do not eat after the larval stage: stored energy is used for metamorphosis, dispersal, and reproduction, all of which are energetically expensive. As an indication of the cost of adult activity, Svensson (1975) showed that *Potomophylax cingulatus* males lost 40% of their mass during the reproductive season. *Neophylax etnieri* has the additional energy requirement of 4–5 mo of diapause, although $<20\%$ of stored lipids are consumed during this period (W. Hill, unpubl. data). The submaximum storage of lipids strongly implies that *Elimia* and *Neophylax* that graze WOC periphyton have reduced abilities to survive stress and contribute to the next generation.

Diminished reproduction is an important means by which limited resources regulate populations. Reproduction was not measured in this study, but size and growth are parameters that should be strongly linked to reproductive success. Female size is a very good predictor of fecundity in most insects (Southwood 1978), and many aquatic insects show fecundity-size relationships (Clifford 1970; Clifford and Boerger 1974; Feminella and Resh 1990). Fecundity in at least one species within the family Limnephilidae (to which *Neophylax* belongs) is linearly correlated with female mass (Svensson 1975). Thus, it seems likely that food-limited size of *Neophylax* translates into food-limited reproduction. There is no broad size-fecundity relationship in snails as there is in insects, perhaps because of variable life-history strategies, but Diamond (1982) reported strong positive correlations between female size and fecundity in *Juga plicifera*, a pleurocerid closely related to *Elimia*. Unless *Elimia* reproduction saturates at very low food levels compared with growth, *Elimia* reproduction was food limited in WOC.

Elimia and other pleurocerids have been characterized as generalists that consume allochthonous matter (e.g. leaves, woody debris) in addition to epilithic microalgae (Mulholland et al. 1985; Hawkins and Furnish 1987; Richardson et al. 1988). Allochthonous matter probably contributes little to *Elimia*'s overall carbon intake, however. The seasonal nature of leaf input reduces its potential to mitigate food limitation (Richardson 1991), especially for long-lived invertebrates such as *Elimia*. Leaves are abundant in WOC only from leaf-fall until the first major spate washes them downstream, a period of usually less than 2 mo (personal observation). As an indication of the relative scarcity of leaves for most of the year in WOC, less than 5% of the snails tagged and recovered in WOC were found on leaves and woody debris. The food quality of leaves and woody debris is also notoriously low (Anderson and Cummins 1979; Pandian and Marian 1986). Leaf additions to a diet of WOC periphyton do not alter *Elimia* growth in the laboratory, and the growth of snails at a site in WOC adjacent to that used in this study actually decreased after leaf-fall (W. Hill, unpubl. data). Thus, it seems unlikely that allochthonous inputs alleviate inadequate autochthonous production.

Both abiotic and biotic factors contribute to inadequate primary production in WOC. Low light limits biomass-specific productivity by periphyton in shaded eastern Tennessee streams during spring and summer when deciduous trees bear leaves (Hill and Harvey 1990), and low nutrient concentrations assume a limiting role after leaf-fall (Elwood et al. 1981; Hill et al. 1992). Grazing by snails at the densities characteristic of these animals in Oak Ridge Reservation streams has negative effects on areal-specific primary productivity even when biomass-specific productivity is released from abiotic constraint (Mulholland et al. 1991; Hill et al. 1992). In WOC, *Elimia* maintains low areal-specific primary productivity by cropping periphyton to low biomass and by depressing biomass-specific primary productivity (Hill et al. 1992). The effect of *Neophylax* on WOC periphyton has not been investigated, but consumption of periphyton by the caddisfly undoubtedly contributes to resource depression and food limitation within the grazer guild during spring.

The threefold greater periphyton biomass in streams without *Elimia* suggests that resource depression by the snail is widespread. Although grazers other than *Elimia* were present in all the streams sampled, the difference in periphyton biomass between streams with and without *Elimia* implies that grazing by species other than *Elimia* was comparatively minor. *Elimia*'s singular effect on periphyton was probably due to its high

standing biomass, rather than an ability to harvest periphyton more efficiently. *Elimia* species crop periphyton inefficiently (Barnese and Lowe 1990), having their greatest effects on upper layers (Hill and Harvey 1990; McCormick and Stevenson 1991). Densities of grazers other than *Elimia* were not quantified in the stream survey, but it was visually obvious that they were unable to attain the standing biomass of the snail. *Elimia*'s apparently unique role in these streams may reside in an ability to escape mortality factors that limit other grazers.

The cause of *Elimia*'s complete absence in six of the surveyed streams was not obvious. Streams lacking *Elimia* were located in five different watersheds and most were adjacent to streams containing high densities of *Elimia*. Major water quality parameters such as temperature, pH, alkalinity, nutrient concentrations, and major cations (W. Hill, unpubl. data) in streams with and without *Elimia* were quite similar. Some of the streams lacking *Elimia* may have gone dry for short periods during the 1988 drought (Bear Creek Tributary, Melton Branch, Pinhook Creek) or suffered chlorine contamination in the past (Northwest Tributary), but others (Fifth Creek, UT Farm Stream) flowed throughout the 1988 drought and were unlikely to have been contaminated. Regardless of the reason(s) behind the snail's absence, periphyton biomass and *Neophylax* condition were assumed greater in streams lacking *Elimia* because of the effects of the snail, and not because some unmeasured factor associated with the snail's absence independently increased periphyton biomass and *Neophylax* condition.

Resource depression by *Elimia* has significant ramifications for other grazers, as indicated by the small size and reduced lipids of diapausing *Neophylax* larvae in streams containing *Elimia*. Exploitative competition is consistent with the evidence of food limitation and extensive dietary overlap between the two grazers. The extent to which competition favors *Elimia*, however, is unclear. *Elimia* may be competitively superior to other grazers, but the effects of *Neophylax* and other grazers on *Elimia* are unknown. Hawkins and Furnish (1987) speculated that snails of the closely related prosobranch genus *Juga* are competitive dominants in western U.S. streams, depressing densities of other invertebrates through a combination of exploitative and interference competition. Although *Elimia* does have significant effects on sessile invertebrates vulnerable to "bulldozing" (Harvey and Hill 1991), interference effects on *Neophylax* were probably negligible due to *Neophylax*'s superior mobility. The very high standing biomass of *Elimia* may be an indication that the competition favors *Elimia*, but as suggested above, differential abilities to avoid top-down factors could also explain *Elimia*'s high standing biomass.

Food limitation may imply that mortality factors are unable to prevent grazers from reaching levels at which they seriously deplete their food resource (e.g. Hairston et al. 1960). The hard shell of *Elimia* and the stone case of *Neophylax* probably function to minimize the effects of both predators and floods. For example, crayfish are important predators on thin-shelled, pulmonate snails (Cowl and Covich 1990; Hanson et al. 1990; Weber and Lodge 1990) and are abundant in eastern Tennessee streams (personal observation). However, *Elimia*'s stout shell seems to discourage extensive predation by crayfish. Snail remnants are uncommon in the gut contents of crayfish from WOC (personal observation). Although the stone case of *Neophylax* is not nearly as durable as *Elimia*'s shell, it probably provides protection from lotic predators such as stoneflies and hellgrammites. *Elimia*'s shell and *Neophylax*'s case also give these grazers relatively high specific gravities, so they are less likely to be swept downstream during storm-related increases in current

(Otto 1976). *Elimia*'s stout shell also provides protection from crushing by small rocks that move on the stream bed during floods. (A. Stewart, pers. comm.). As an example of *Elimia*'s resistance to floods, snail densities did not decline significantly after a summer thunderstorm increased discharge in WOC 40 times (W. Hill, unpubl. data).

The extent of grazer food limitation is unknown. I hypothesize that food limitation is prevalent within grazer guilds in the many North American headwater streams that feature high densities of prosobranch snails and low primary production. Although Stock and Ward (1989) concluded from estimates of periphyton and snail production that *Elimia* was not food limited in a northern Alabama stream with snail densities and primary production rates similar to those in WOC (Boston and Hill 1991), the empirical demonstration of severe food limitation in this study suggests that Stock and Ward's (1989) calculations are in error. A growing number of studies imply that food limitation is also common without prosobranch snails. Food-limited growth and exploitative competition have been suggested for the mayfly *Ameletus validus* in a California stream (Hill and Knight 1987), the caddisflies *Glossosoma nigrum*, *Leucotrichia pictipes*, and *Psychomyia flavida* in a Michigan stream (Hart 1987; Hart and Robinson 1990), and the caddisfly *Helicopsyche borealis* in another California stream (Lamberti et al. 1987; Feminella and Resh 1990). Such resource dependence is contrary to models in which herbivore populations are susceptible to control by predators (e.g. Hairston et al. 1960; Carpenter et al. 1987; Power 1990) and lends relevance to studies of primary production in streams. Secondary production in streams where grazers comprise a significant portion of the benthos should closely track physical/chemical factors that limit primary production.

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Assessment of Crab Limb Regeneration as an Assay for Developmental Toxicity

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The regeneration of a cheliped that is autotomised at the final larval stage, the megalopa, of the mud crab *Rhithropanopeus harrisii* (Gould) forms the basis of an assay for developmental toxicity. Cheliped regeneration is followed through to the third juvenile crab stage; a regenerate that is approximately two thirds full size normally emerges at the moult to the second crab, and full size is attained at crab 3. The absence of a regenerate at crab 2, or a regenerate that is smaller than normal and/or malformed, is scored as abnormal regeneration. Other parameters examined in this assay include survival and duration of development. The assay is of comparatively short duration (approximately 2 wk), and survival is high following autotomy under optimal conditions. Four insecticides and a herbicide have been tested in the assay at lethal and sublethal concentrations. Of these compounds, methomyl, carbofuran, and alachlor induced abnormal regeneration whereas cypermethrin and RH 5849 did not affect regeneration at the concentrations tested. Although reproducibility of results needs improvement, crab limb regeneration is otherwise a practical assay for developmental toxicity.

La régénération d'une patte préhensile autoamputée à la fin du stade larvaire (mégaloïpe) chez un crabe de vase à pinces blanches, *Rhithropanopeus harrisii* (Gould), est à la base d'une expérience sur la toxicité au cours du développement. On a observé tout le processus de régénération de la patte chélicoïpe jusqu'au troisième stade juvénile de développement du crabe; un régénérat dont la taille égale environ les deux tiers de celle d'une patte préhensile de spécimen adulte apparaît normalement à la suite de la première mue, le régénérat atteignant sa taille normale à la suite de la deuxième mue. L'absence de régénérat à la première mue, ou la présence à cette phase d'un régénérat de taille inférieure à la normale ou difforme, est considérée comme étant une régénération anormale. Parmi les autres paramètres examinés dans le cadre de ces expériences se trouvent la survie et la durée du développement. L'expérience est de durée relativement courte (environ 2 sem) et le taux de survie est élevé à la suite d'une autotomie dans des conditions optimales. Au cours de ces essais, on a testé quatre insecticides et un herbicide à des concentrations létales et sublétales. Les composés qui ont provoqué une régénération anormale sont le méthomyl, le carbofuran et l'alachlor, tandis que la cyperméthrine et le RH 5849 n'ont pas nui à la régénération aux concentrations testées. La reproductibilité des résultats pourrait certes être améliorée; cependant, la régénération de pattes de crabe constitue, sous les autres aspects, une méthode pratique pour tester l'effet de la toxicité sur le développement.

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The purpose of this paper is to assess the practicality of using limb regeneration in megalopal and juvenile crabs as a sublethal bioassay of developmental toxicants in the marine environment and as a more general model of developmental toxicity. Among the more important considerations of practicality are (1) availability of the test animal, (2) cost of the assay, (3) duration of the assay, and (4) reproducibility. With the possible exception of (4), where improvements may still be made, the limb regeneration assay meets these criteria.

A marine developmental bioassay may be applied potentially in three ways: (1) as a sublethal bioassay for toxicants introduced into the marine environment, (2) as a more general model of developmental toxicity, and (3) to screen for potential teratogens to higher organisms, including humans. The latter concept is gaining increasing acceptance and parallels both the growing interest in marine organisms as models in biomedical research (Marine Life Sciences Workshop 1989) and the search

for alternatives to higher vertebrates in toxicity testing (Goss and Sabourin 1985; Goldberg and Frazier 1989).

In recent years, a number of aquatic organisms (vertebrates and invertebrates) have been proposed to test for developmental toxicity (Duffus et al. 1984; Weis and Weis 1987). The embryonic phase of an organism's life cycle is viewed as being particularly sensitive to developmental toxicants. The regeneration of tissues, a process akin to embryogenesis (Weis and Weis 1987), has also been used as a bioassay for teratogens. Particularly noteworthy is the bioassay developed by Weis and co-workers (Weis 1976; Weis and Mantel 1976; Weis et al. 1987a, 1987b) involving the regeneration of an adult crab limb.

Decapod crustaceans have the ability to autotomise their appendages and subsequently regenerate them at most stages of their life cycle (McVean 1982). Limb regeneration has received considerable attention and has been reviewed recently by Hopkins (1988). The comparative simplicity of the process

and the ability to make noninvasive observations on the process of development make it attractive for studies of developmental toxicity. The regenerated tissues comprise the exoskeleton and underlying epidermis, nerve, and sensory tissue (e.g. setae), chromatophores, muscle, and blood vessels. The limb develops folded within a cuticular sac, the limb bud (Bliss 1960; Lumb et al. 1991). In the adult crab, the limb bud is large enough to permit accurate *in situ* measurements during regeneration. The relation of the length of the limb bud to the width of the carapace gives an index of regeneration (Bliss 1956). This index has proven useful in assessing the effects of xenobiotics on the regeneration process. In addition to an effect on size, more pronounced effects in the form of malformations have been noted for a range of toxicants (Weis 1976; Weis and Mantel 1976; Weis et al. 1987a, 1987b).

Autotomy is not confined to the adult crab. The final larval stage, the megalopa, also has this ability and is able to regenerate lost appendages which emerge at either the first or second juvenile crab depending on the timing of autotomy and the crab species (Costlow 1963; McConaughy and Costlow 1980, 1987; Clare and Costlow 1989). As the larval stages of an organism are, generally speaking, more sensitive to pollutants than the adult, limb regeneration at the megalopal stage has the potential to be used as a sensitive sublethal bioassay of environmental contamination.

This paper describes the cheliped regeneration bioassay and illustrates its strengths and shortcomings by presenting the results obtained with four pesticides and a herbicide. Some of the preliminary findings from this study have been reported elsewhere (Clare and Costlow 1989; Costlow 1990).

Materials and Methods

Source of Animals and Their Maintenance

Adult, gravid, mud crabs (*Rhithropanopeus harrisi* (Gould)) were collected from plastic crates or metal box traps, both containing oyster shells, at two sites: the Neuse River and the South River, North Carolina. In the laboratory, they were placed individually, together with one oyster shell, into 19-cm-diameter, glass Carolina culture dishes containing 600 mL of 20 parts per thousand (ppt) filtered (5 μ m) seawater. The crabs were kept in an environmental cabinet operating at 25°C and a photoperiod of 12 h light : 12 h dark. The seawater was changed daily, at which time the crabs were fed *ad libitum* with newly hatched *Artemia* sp. (Sanders; Great Salt Lake). Upon hatching, the larvae were transferred to clean bowls using a wide-bore glass pipette. The larvae were maintained under the same conditions as the adult crabs until they reached the megalopal stage, whereupon autotomies were performed.

Autotomy

The right cheliped was selected as the limb for autotomy. For the operation, a megalopa was placed on a depression slide and immobilised by pipetting off excess seawater. The merus of the selected limb was then pinched gently with watchmaker's forceps. Only those animals that readily autotomised their cheliped were used for subsequent studies (Clare and Costlow 1989). Animals were operated on within 17 h of the moult to the megalopal stage which for the purposes of this study was assumed to occur at 00:00. The importance of the timing of autotomy to subsequent regeneration has been discussed by McConaughy and Costlow (1980). An experienced operator can perform approximately one autotomy per minute, taking about

2 h to perform autotomies sufficient for a single assay. Survival has been shown to be comparable for operated and control animals (cf. Clare and Costlow 1989). For good survival, it is imperative that the autotomy membrane (Hopkins 1988) is not damaged during autotomy, as this membrane prevents loss of haemolymph.

Test Compounds

All compounds were technical grade and of the highest purity available. The carbamate insecticide methomyl and the insect growth regulator RH 5849 were the kind gifts of E. I. DuPont de Nemours Co., Inc. (Wilmington, DA), and Rohm and Haas (Spring House, PA), respectively. The carbamate carbofuran was obtained from Chem Service (West Chester, PA) and the herbicide alachlor and the synthetic pyrethroid cypermethrin from Crescent Chemical Co., Inc. (Hauppauge, NY).

Assay Conditions

Range finding tests were performed for all compounds to establish the definitive concentration (nominal) range to be used in the regeneration bioassay. Three to five replicates of each concentration were used in the bioassay with 6–10 megalopae in each replicate, except for alachlor and RH 5849 where a total of 20 and 10 megalopae, respectively, were autotomised at each concentration. Depending on the numbers available, megalopae from one female or a pool from two or more females were autotomised. Pooled animals were assigned randomly to the different treatments. The appropriate control series was run for each compound: a 20 ppt seawater control was employed for methomyl and an acetone control for the remaining compounds (alachlor, carbofuran, cypermethrin, and RH 5849) that required a carrier solvent. Acetone was present in the test and control solutions at a concentration of 1 ppt. Previous studies have established that this concentration of acetone has no noticeable effects on larval development (Bookhout et al. 1976).

The autotomised megalopae were placed individually in 15-mL glass vials (Fisher Scientific Co.) containing 5 mL of the appropriate solution and covered loosely to reduce evaporation. The megalopae were maintained in an environmental cabinet at 25°C and a 12 h light : 12 h dark photoperiod to the third juvenile crab. Each day, the megalopae or crabs were transferred with a wide-bore glass pipette to clean vials containing freshly prepared solutions to which one drop of newly hatched *Artemia* sp. nauplii was added.

Observations

Daily records were kept of the condition of each animal: whether it was alive, moribund, or dead, whether it had or was about to moult, and whether a regenerate had appeared. If a regenerate had appeared, it was compared with the contralateral cheliped and its gross morphology recorded. For the purposes of this study, abnormal regeneration is characterised as the failure of a regenerate to appear at the expected ecdysis (second crab stage; Clare and Costlow 1989) or a regenerate that is smaller than normal and/or malformed. Survival in the regeneration assay is expressed as the percentage of megalopae that successfully complete development to the third crab stage.

Statistical Analysis

Data expressed as percentages were arcsine transformed prior to statistical analysis. Alachlor regeneration and survival data

and RH 5849 survival data were analysed using a *G* test for independence (Sokal and Rohlf 1981). The remaining data were analysed according to Williams (1971, 1972) as recommended by Gelber et al. (1985).

Results

Survival

For control megalopae, survival is normally in excess of 80% and ideally should approach 100%, although some variation can be expected in the progeny of different females. Occasionally, however, survival is comparatively low. The results presented for alachlor illustrate one incidence of poor survival for controls. Obviously, if survival is too low, it becomes difficult to distinguish effects of the test compound

on crab development. Based on the results of range-finding tests and subsequent regeneration bioassays (Fig. 1), the toxicities of the compounds tested can be ranked as follows: cypermethrin > carbofuran > methomyl > alachlor > RH 5849. The animals were particularly sensitive to the toxicants at ecdysis from the megalopa to the first juvenile crab (cf. Clare and Costlow 1989).

Duration of Development

Protracted development was noted for methomyl, cypermethrin, and alachlor. The effect is particularly marked when the cumulative duration of the individual stages of development is calculated, i.e. the period of development from the megalopa to the third crab stage (Table 1). It is also apparent that there

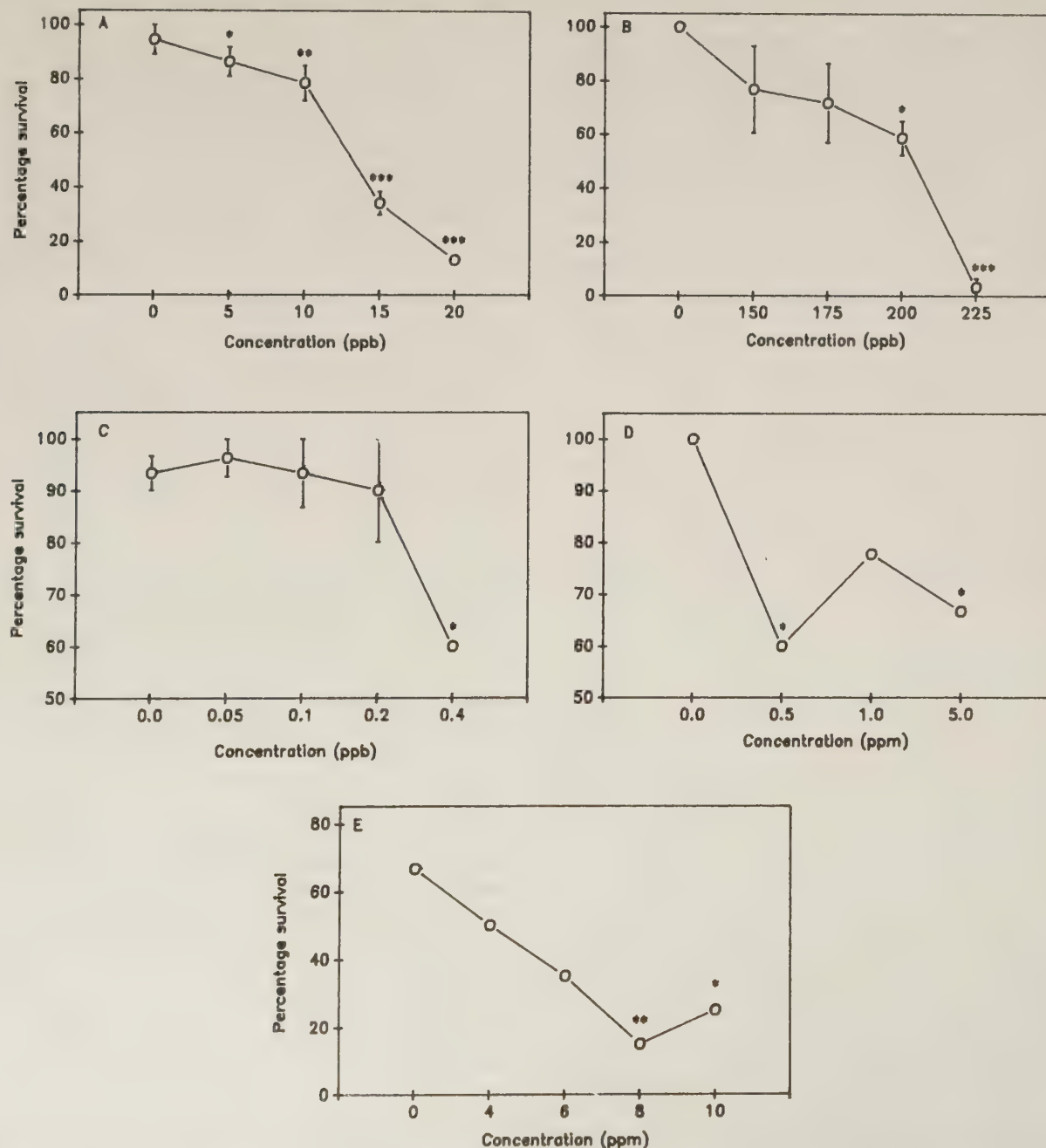


FIG. 1. Effect of the test compounds on the survival of autotomised megalopae to the third juvenile crab stage compared with controls. (A) Carbofuran; (B) methomyl; (C) cypermethrin; (D) RH 5849; (E) alachlor. Difference from controls significant at **P* < 0.05, ***P* < 0.01, and ****P* < 0.005. Note different scale of concentration in Fig. 1D and 1E.

TABLE 1. Duration of development from the megalopa to crab 3 in the presence of test compounds.

Compound	Concentration	Duration of development (days $\pm$ SE)	Significance (compared with control)
Methomyl	0 ppb	13.83 $\pm$ 0.19	
	150 ppb	16.06 $\pm$ 0.34	$P < 0.005$
	175 ppb	16.28 $\pm$ 0.14	$P < 0.005$
	200 ppb	16.77 $\pm$ 0.47	$P < 0.005$
Carbofuran	0 ppb	16.24 $\pm$ 0.22	
	5 ppb	17.77 $\pm$ 1.26	n.s.
	10 ppb	16.81 $\pm$ 0.68	n.s.
	15 ppb	17.95 $\pm$ 0.97	n.s.
Cypermethrin	0.0 ppb	15.76 $\pm$ 0.09	
	0.05 ppb	15.71 $\pm$ 0.25	n.s.
	0.1 ppb	16.99 $\pm$ 0.45	$P < 0.05$
	0.2 ppb	17.27 $\pm$ 0.30	$P < 0.05$
	0.4 ppb	16.78 $\pm$ 0.05	$P < 0.05$
RH 5849	0.0 ppm	14.60 $\pm$ 0.22	
	0.1 ppm	14.33 $\pm$ 0.33	n.s.
	1.0 ppm	14.14 $\pm$ 0.26	n.s.
	5.0 ppm	15.67 $\pm$ 0.49	n.s.
Alachlor	0 ppm	15.83 $\pm$ 0.48	
	4 ppm	16.60 $\pm$ 0.52	n.s.
	6 ppm	18.14 $\pm$ 0.74	$P < 0.05$

are differences in the duration of development between control animals in the different experiments.

Cheliped Regeneration

Regeneration proceeded in a manner similar to that described for adult crabs (Hopkins 1988). The cheliped developed folded within the limb bud and normally emerged fully formed, although some two thirds full size, following ecdysis to the second crab stage.

Three of the compounds tested in the bioassay, methomyl, carbofuran, and alachlor, caused abnormal cheliped regeneration but at widely different concentrations depending on the toxicant (Fig. 2). Malformed regenerates ranged from simple stumps with little or no form to chelipeds that were bent the wrong way or that possessed a comparatively minor fault such as a twisted dactyl. Regenerates were not measured; they were compared visually with the contralateral cheliped. The judgement of size was thus rapid but entirely subjective. Carbofuran was the most potent inducer of abnormal regeneration, effects being noted at 20 parts per billion (ppb). For methomyl, the lowest concentration to cause abnormal regeneration was 150 ppb, while 100% abnormal regeneration occurred in 225 ppb. Much higher concentrations of alachlor (6–10 ppm) were required for abnormal regeneration. Regeneration was not affected by RH 5849 or cypermethrin. Although the absence of a regenerate was occasionally noted in control solutions (Fig. 2B), no malformed regenerates were noted in this study.

Discussion

An examination of the data for survival and duration of development of control animals reveals inconsistencies between assays. We believe that this variability results in part from the use of different female crabs collected at different sites, differences in the physiological status of crabs collected at different times of the year, and differences in laboratory water quality. Much of this variability may be circumvented by the development of a laboratory-reared genetic line. However, this

advantage must be offset against a possible increased sensitivity to toxicants and consequent decrease in environmental realism. Further consistency in survival may also result from the use of "pristine" field-collected seawater (e.g. of oceanic origin) or artificial seawater that is prepared from nanopure water.

A common effect of xenobiotics on crustaceans is protracted development. Wells (1976) and Epifanio (1979) suggested that this sublethal effect is a generalised response to stress. However, there are a few exceptions that contradict this theory (Christiansen et al. 1978; Johns and Pechenik 1980). In the present study, neither carbofuran nor RH 5849 significantly ($p > 0.05$) prolonged development. The latter result is not surprising, since RH 5849 acts as an ecdysone (arthropod moulting hormone) agonist in insects (Wing 1988) and possibly crustaceans (Clare et al. 1992). On this basis the concentrations of RH 5849 tested in this study may have been too low to shorten development.

Abnormal regeneration and protracted development are sublethal effects at the megalopal and juvenile stages of mud crab development. Nevertheless, the regeneration bioassay in its present form is not meant to be a sensitive indicator of general environmental quality. In fact, compounds that display sublethal toxicity in the regeneration bioassay are acutely toxic at lower concentrations to earlier developmental stages. For example, the 24-h LC_{50} for carbofuran to the second zoeal stage of *R. harrisii* is 9.5 ppb (A. S. Clare, unpubl. data). In other words, it is unlikely that the megalopal stage of development would have been reached at the concentrations tested in the regeneration bioassay.

Cheliped regeneration may be a sensitive indicator of developmental toxicity. We have examined the process of regeneration in the progeny of different females from two sites: South River and Neuse River, North Carolina. Extensive observations on megalopae and juvenile crabs lead us to believe that malformed regenerates do not occur under established optimal conditions of culture. However, the data are at present insufficient to make comparisons with other bioassays of developmental toxicity. In particular, further studies on the effect of known teratogens on cheliped regeneration are required.

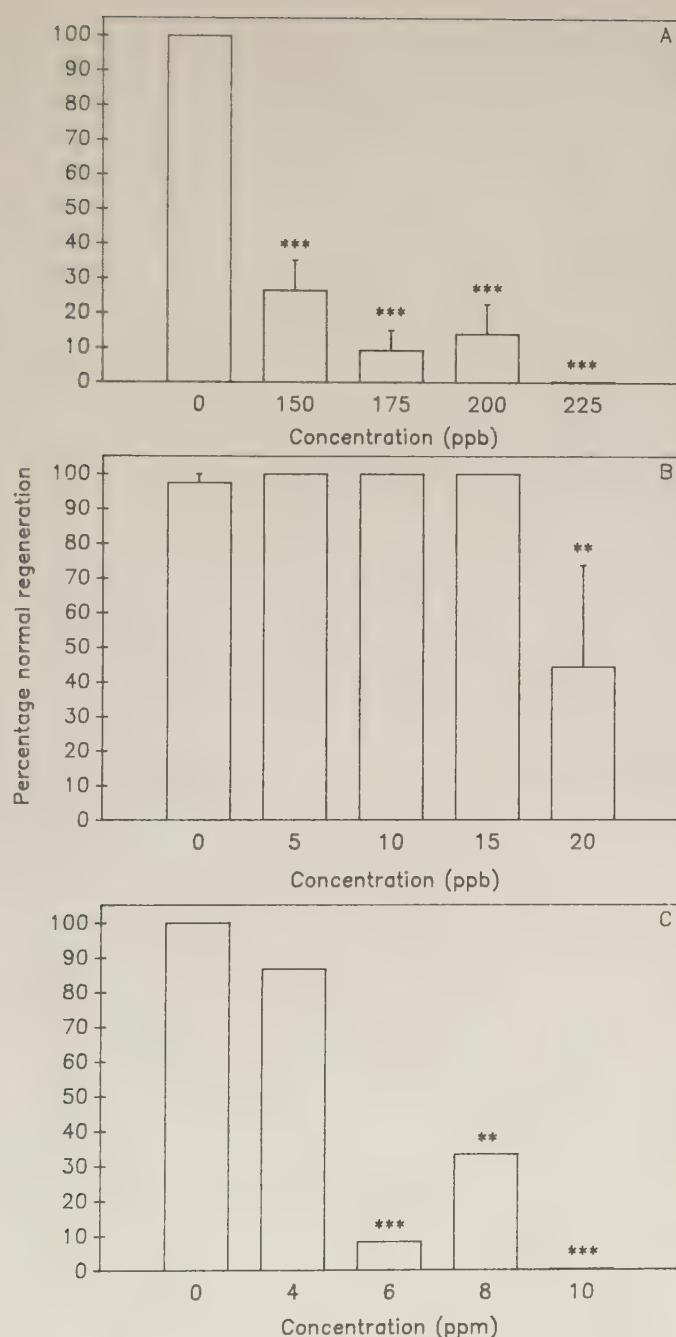


FIG. 2. Incidence of abnormal regeneration in the presence of exogenous pesticides. (A) Methomyl; (B) carbofuran; (C) alachlor. Difference from the control significant at $*P < 0.05$, $**P < 0.01$, and $***P < 0.005$. Note different scale of concentration in Fig. 2C.

Several features of *R. harrissii* and the regeneration assay lend themselves to the study of developmental toxicity: (1) the mud crab is a common estuarine organism and has a wide distribution; (2) the biology of *R. harrissii* has been studied extensively; (3) the adult crabs are easily maintained in the laboratory, and optimal conditions for complete larval development with high survival are known (Costlow et al. 1966); (4) although this crab is a seasonal breeder in the field, its reproduction and development can be manipulated in the laboratory (Bookhout et al. 1984) so that larvae are available throughout the year; (5) their small size, especially of the larval and juvenile forms, enables large numbers of larvae of known parentage to be maintained so that critical statistical analyses can be performed; (6) rapid development of the larval and juvenile forms means that the

regeneration process is complete (i.e. the limb is full size) in approximately 2 wk at 25°C, i.e. rapid development also means that complete life cycle and multigeneration studies are practicable; (7) crab limbs can be observed by noninvasive techniques; and (8) the cheliped, while composed of epidermis, cuticle, muscle, nervous, and sensory tissue and the whole perfused with haemolymph, is a comparatively simple structure. Notwithstanding these advantages, the early developmental stages of *R. harrissii* and other crabs have not been utilized routinely in environmental quality assessment and monitoring for several reasons: a lack of test method standardization (Gentile et al. 1984); it has only recently been shown that they can be exposed to toxicants in flow-through conditions (Singer et al. 1990), they are perceived to be, and are in many cases, very labour intensive to work with, there are few skilled persons in the field of aquatic toxicology promoting their use, and control mortalities can be unacceptably high.

In summary, cheliped regeneration has been used to test the developmental toxicity of five compounds. Of these compounds, the carbamate insecticides were the most potent teratogens, but the site and mechanism of the developmental lesion were not ascertained. Histopathological studies of cheliped regeneration are now in progress. On the basis of these studies, it may be possible to develop a bioassay of developmental toxicity that is sensitive to teratogens, is applicable to the marine environment, especially if used as an in situ test, and may also serve as a more general model of developmental toxicity.

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LETTERS AND COMMENTS LETTRES ET COMMENTAIRES

Aquatic Invertebrates and Climate Change: A Comment on Walker et al. (1991)

Walker et al. (1991) present sweeping generalizations on the role of climate in *directly* determining geographic distributions of aquatic invertebrates and their utility as paleoclimatic indicators. Our concerns are not whether chironomids can be useful indicators of past climate or climate change, but with the *implied direct, overriding linkage* between temperature (as a proxy for "climate") and species range. Lakes and wetlands represent one of the most important repositories of paleoenvironmental information, and so it is important to understand how to use the biological record preserved in them for paleoclimatic reconstructions.

The interpretations of Walker and Mathewes (1987a, 1987b) and Walker et al. (1991) reflect insufficient differentiation between *patterns* of correlated biotic change and associated causative *processes* (sensu Birks 1986) in paleolimnological space and time. According to Ritchie (1986), "multiple relationships controlled by multiple causes operate at varying scales of time and space." Schindler et al. (1990) found that some lakes may have responded to external meteorological factors during historical time. Forester (1987), however, emphasized the complex relationships between climate and lake hydrogeochemistry and the responses of aquatic invertebrates in low-latitude lake basins to these many environmental changes. How do these external factors affect internal physical and biotic characteristics of lakes, if at all? Were past variations in climate intense and long enough to affect lake biota similarly? Are these meteorological perturbations manifest in the fossil records of lake sediments? Was the physical, chemical, and biological structure of Holocene lakes such that most aquatic biota would experience variations in climate of sufficient magnitude and intensity to require a shift in their geographic limits?

Effects of climate

Orbital oscillations between the earth and the sun were the main forces driving world climate during the Quaternary (Imbrie and Imbrie 1980). The resulting climatic and environmental changes over the last 20 000 yr have necessarily had strong effects on the biota. When conditions in a habitat change, organisms can move, adapt, or go extinct. The first option is recorded as range migration, a change in the centre of abundance, or range extension or contraction. Pollen analyses show that tree populations tracked environmental changes induced by fluctuations in climate by moving location rather than adapting in situ (Bartlein and Prentice 1989; Bennett 1990). Evidence that microevolutionary adaptation or speciation was associated with climate change has been demonstrated by Hann (1982) and Shan and Frey (1983). Extinctions as a consequence of drastic climate change are clearly and abundantly recorded in the paleontological literature.

Climate may have ultimate control over the abundance and distribution of taxa, but other factors play important roles at different temporal and spatial scales (Glantz 1990). Species

abundances and distributions are dependent variables influenced by two sets of independent variables, the physical environment and other species (Diamond 1986). The current controversy is based on determining which predominates, when, and how much of the time. Are communities structured by interaction between species, or is the assemblage a consequence of the independent reactions of individual species to environmental (including climatic) factors? We concur with Prentice (1986) that "as spatial and temporal frames of observation are diminished and resolution increases, biotic processes must eventually come to dominate."

The stressful effects of climate change on a species will vary depending on the rate and dimension of change (Davis 1990; Parsons 1990), the predictability of the habitat, and whether a central or marginal population of the species is impacted (Ritchie 1986). The aquatic ecosystem, and particularly the deep-water benthos, is well buffered from temperature change (Forester 1987; Walker and Mathewes 1987a), probably within a temporal scale of 10^2 – 10^3 yr. Aquatic organisms thus respond more slowly to climatic change than terrestrial vegetation, contrary to the suggestion made by Walker et al. (1991). Core populations near their environmental optimum typically are controlled by density-dependent processes, where biotic factors are important. In contrast, density-independent processes take on a larger significance in marginal populations, as do conditions of the physical environment. It is for this reason that persistent range extensions and contractions of fringe populations (of stenothermal species especially) are so critical to climatic interpretations (Coope 1977, 1987). But does absence of a climatically sensitive taxon from a marginal locality signal that climatic conditions are beyond the physiological tolerance of that taxon (Davis 1986)? Or is the absence a function of the vagaries of chance (or sampling error) when dealing with a rare species at its distributional limits? Unfortunately, little is known as yet about the environmental parameters that delimit the species ranges in fossil assemblages or modern communities.

Climatic inferences based upon species presence/absence in an assemblage also need to be interpreted very carefully. For example, sibling species pairs, delimited physiologically and reproductively isolated, are known in the Cladocera (Hann 1982; Shan and Frey 1983) but are indistinguishable as fossil remains. These north-south species pairs seem to have differentiated in response to past climatic change, exemplifying the middle option, that of local adaptation rather than migration or extinction, but merging of such distributions into one range could distort or lead to misinterpretation of climatic changes.

Smol et al. (1991) suggest that "direct inferences of past temperatures [and other environmental parameters] can be made where the thermal optima and tolerances of species are known". Delorme's (1989) paleoclimate model for ostracodes currently is the only such model available that is based on an adequate body of contemporary autecological information from which we can extrapolate past environments and suggest paleoenvironmental reconstructions with a reasonable degree of confidence.

According to Bryson (1985), "climate is multidimensional (a vector), not a single scalar datum", yet Walker et al. (1991) use a *single surface water temperature reading per lake*, taken on an unspecified date from a source that is not clearly identified, as representative of "climate" for a given lake. This is inappropriate. Even if climate in general could be implicated in determining the range of a species (e.g. Patalas 1990), the crucial aspect of climate may differ from one species to another. For instance, mean annual air temperature, mean summer air (water) temperature, maximum summer air (water) temperature, minimum winter (air) temperature, duration of open-water season, and mean (minimum) number of degree-days are all components of climate that may play a role in delineating a species' range, as mediated through life history/life cycle requirements. Those aquatic invertebrates with an aerial adult life stage (e.g. Chironomidae, Trichoptera, Ephemeroptera, and Coleoptera) may face critical climatic requirements different from those with an entirely aquatic life cycle.

Walker et al. (1991) have applied their transfer function, built using Labrador data, to chironomid assemblages from a suite of lakes in British Columbia. Despite the very poor fit of the British Columbia data to the model, they suggest that this was a "rigorous test" of that model. We disagree: not only are the data not immediately available and the statistics questionable, but there is considerable risk involved in extrapolating from one region to another in North America let alone extrapolating from a European ecological database to make inferences on climatic response from biotic data.

Chironomidae as quantitative indicators of past climate change?

Many of the principles to be considered in any study using aquatic invertebrates as indicators of climatic change (Warner and Hann 1987; Warwick 1989) were not considered by Walker et al. (1991). These authors have constructed a model describing the relationship between temperature and chironomid species distributions, based on a single surface water temperature reading taken by Clair et al. (1982) over the period 20 July – 23 August 1979. How meaningful can a single measurement of temperature be in establishing a climatic determinant for chironomid distributions and community composition (Shuter et al. 1983)? Walker et al. (1991) acknowledge that "temperature and water chemistry measurements were obtained during a single visit to the lakes in 1979. They provide an imperfect assessment of local environmental differences, including temperature conditions [especially when] local climatic conditions are especially prone to annual variations".

The authors have chosen to use complex statistics on a very small data set, encompassing a broad range of lake types and sizes. Their goals would have been better achieved by selecting either lake basins with similar geological, limnological, and biological settings or a larger number of each lake type. The representativeness of a single sample of the whole lake biota and lake habitat, especially when some samples (even of the 26 remaining from the original total of 33 samples) contained a limited number of individuals, also is open to question. And how representative of a lake basin's substrata is one sediment core, especially for organic content determination?

Walker et al. (1991) claim that temperature explains most of the variance (22%) in the environmental variables, yet both lake depth and Secchi depth contribute 20%. This slim margin makes the choice for the forward selection procedure somewhat arbitrary (see their table 2, values not as stated in text?). These results do not support the conclusion that surface temperature

is the dominant variable determining species composition; to the contrary, the evidence which Walker et al. (1991) present buttresses our contention that species composition is determined by a multiplicity of environmental variables.

Depth, transparency, and turbidity have previously been shown to have definitive effects on chironomid communities (Warwick 1980, 1989). Walker et al. (1991) themselves confirm that temperature and lake depth are highly correlated with axis 1 in their analysis. To single out one as being of greater explanatory significance on the basis of their statistical analysis is unwarranted. Therefore, neither null hypothesis 1 nor 2 in Walker et al. (1991) can be rejected unequivocally. Axis 2 also accounts for 22% (versus 37% for axis 1) of the variance, but is weakly interpreted.

The support for the third null hypothesis, no correlation between relative abundance of fossil chironomid taxa and sediment organic content, is spurious in our opinion. "Organic matter concentrations are invariably influenced by mineral sediment dilution" (Warwick 1980), but Walker et al. (1991) admit that no sediment accumulation rate information was available for any lake. They also admit that evidence exists to support the correlation between chironomid taxa and sediment composition, but claim that the relationship is weakened because of the confounding effects of water temperature or depth. Neither McGarrigle (1980) nor Winnell and White (1985), whom Walker et al. (1991) quote as evidence, make direct reference to correlations between substrate differences, water temperature, or depth.

The null hypotheses are explicit and falsifiable, but in the end they leave all three inadequately tested. None of the three null hypotheses deals with turbidity. Although lake depth does strongly influence chironomid community composition, it is not a direct predictor of any aspect of climate. These oversights point to the problem of using single variables as indicators of complex, multidimensional environmental factors.

Walker et al. (1991) treat littoral and profundal chironomid taxa without differentiation as input to a transfer function to predict surface water temperature and, by inference, to predict "climate". However, "knowledge about the water temperature of the hypolimnion (and by extrapolation, deep-water fauna) in any type of northern lake provides little information about seasonal air-temperature variation" (Forester 1987; Walker and Mathewes 1987a) and, by inference, little about climate in general. Any relationship observed between surface water temperature and chironomid remains must depend on proportional representation of littoral taxa in deepwater sediments. Although Walker et al. (1991) cite Frey (1988) to support offshore deposition of littoral invertebrate remains, Frey (p. 188), in actual fact, states specifically that "the extent to which chironomid remains are moved offshore is still not completely resolved. . . In the middle of a large, deep lake there should be only occasional head capsules of littoral species in the sediments. Iovino (1975) concluded that offshore transport of head capsules is strongly influenced by surface area of the lake, as affecting wind disturbance, and the volume of deep water, and hence its success declines sharply with depth and distance from shore". Similarly, Wiederholm (1979, p. 255), whom Walker et al. (1991) also quote, states that "cores from a more central part of the lake may show a lower proportion of littoral and sublittoral taxa". The littoral, sublittoral, and profundal components of the chironomid fauna must be evaluated independently with respect to impact of physical, chemical, and biotic factors over time in order to tease apart the significant

environmental influences and improve the utility of invertebrate microfossils in paleoecological reconstructions.

As they themselves admit, Walker et al. (1991) have been snared in the trap of circular reasoning in testing their Labrador chironomid-temperature model. Transfer functions should be validated using *independent modern data sets* and not the *same data set used to develop the model initially*. The near perfect relationship between actual and predicted temperatures (their fig. 3) not only is an example of spurious correlation, but the 3–5°C range in temperature predicted in the model may not provide a fine enough discrimination for paleoclimatic reconstruction when this range may represent the entire amplitude of change over thousands of years. And yet they deem their model acceptable despite the poor fit with the British Columbia lake samples (their table 6) and the weak correlation ($r^2 = 0.53$) between predicted temperatures and altitude. In conclusion, we feel that Walker et al. (1991) have come a very short way in their attempt (a) to infer paleoclimate directly from fossil chironomids and (b) to develop and calibrate a tool for precise quantitative paleotemperature estimation. These questions must be addressed much more rigorously before chironomids, and especially the Walker et al. model, are widely and indiscriminately used in paleoclimatic reconstructions — Brenda J. Hann, *Department of Zoology, University of Manitoba, Winnipeg, Man. R3T 2N2, Canada*, Barry G. Warner, *Department of Geography and Quaternary Sciences Institute, University of Waterloo, Waterloo, Ont. N2L 3G1, Canada*, and William F. Warwick, *National Hydrology Research Institute, Aquatic Ecology Division, 11 Innovation Blvd., Saskatoon, Sask. S7N 3H5, Canada*. (JB143)

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Aquatic Invertebrates, Climate, Scale, and Statistical Hypothesis Testing: A Response to Hann, Warner, and Warwick

Hann et al. appear unwilling to accept our evidence indicating that chironomid distributions are strongly linked to water temperature and that chironomids can be useful indicators of past climate. Their comment continues a 5-yr debate on this subject (Warner and Hann 1987; Walker and Mathewes 1987; Warwick 1989; Walker and Mathewes 1989a, 1989b), which compelled us to undertake the analyses presented in Walker et al. (1991b). Their present comment, in our opinion, contains errors, unsubstantiated concerns, and some misunderstandings and mistrust of our statistical treatments. We emphasise that the statistical methods Walker et al. (1991b) employed, and that Hann et al. consider “complex” and “questionable”, are becoming standard in many quantitative palaeolimnological studies (see Battarbee 1991 for a review).

In our view, the key error of Hann et al. is a lack of appreciation of the importance of scale in determining whether or not a particular environmental variable significantly influences a particular species' distribution. For example, without contemplating its full implications, they write "Climate may have ultimate control over the abundance and distribution of taxa, but other factors play important roles at different temporal and spatial scales (Glantz 1990)". We wish to emphasize two points: (1) this statement is equally true for all biological palaeoclimatic indicators, terrestrial or aquatic, and (2) our findings are in complete accordance with this statement; at the broad scale, climate is exercising ultimate control.

If we were to expand our database to include lakes spanning an even greater climatic range (see Walker 1991), the influence of climate can be expected to explain an increasing proportion of the variance. If the scale were contracted to the point where all the lakes studied received the same thermal input, any effect of climate would not be discernible, and depth and other local factors, together with biotic processes, would inevitably assume dominance. As Wiens et al. (1986, p. 145–146) conclude, "Some of the most vociferous disagreements among ecologists arise from differences in their choice of scale. Those who study the long-term dynamics of a particular community at a single site often reach very different conclusions from those reached by individuals conducting short-term studies of similar communities at many different sites distributed over a large area... All too often ecologists accustomed to working on a certain scale react to these differences by disparaging the value of work at another scale, rather than by attempting to reconcile the differences or to benefit from the insights of that work. Short-term, broad-scale studies are often decried as superficial, addressing only patterns rather than processes, producing artifactual findings, and impotent to discern critical factor X."

Because the environmental changes that we are primarily interested in at the end of the last glaciation involved shifts in climate and atmospheric circulation patterns on an enormous scale, our focus should not be on small-scale processes. At midlatitude sites, late-Quaternary climatic changes did not involve small variations in an existing climate, but instead abrupt shifts, including dramatic oscillations between arctic and temperate climates (e.g. Broecker et al. 1985).

As aquatic ecologists have expanded the spatial scale of their work, they have repeatedly recognized that climatic conditions and temperature are amongst the best predictors of species composition, diversity, and productivity (e.g. Kalff 1991; Mouton 1990; Patalas 1990). Using different statistical techniques than we use, Rossaro (1991) independently concluded that chironomid species distributions are inextricably linked to water temperature. In fact, a strong chironomid–climate link has been recognized since, at least, the early work of Brundin (1958).

Hann et al. express concerns about intralake variability in species assemblages (i.e. how representative is a single sample?). Where nearby lakes of similar size have been studied palaeolimnologically (e.g. Walker and Paterson 1983), very similar faunas are found in contemporaneous early postglacial sediments. If large variations in fauna occurred near the centre of a single lake, these interlake similarities would rarely be evident. The interlake variation among deepwater coring sites will be at least as large as the intralake variability. The fact that littoral assemblages differ from profundal assemblages is irrelevant because if a single core is taken from a lake, it should always be taken near the point of maximum depth. In diatom work, it is recognized that littoral assemblages differ from profundal assemblages, but these variations in fossil floral com-

position generally have little impact on the palaeo-pH inferences if the assemblages are contemporaneous (e.g. Charles et al. 1991). The important question is not whether variations in fauna occur from site to site near the centre of the lake, but rather, if such differences exist, do they significantly influence the temperature inferences?

It is surprising to hear Hann et al. raise the issue of variability; Hann and Warner (1987) and Warwick (1980) did not examine multiple cores. Birks (1973) and Wainman and Mathewes (1990) demonstrated enormous variability in assemblages of plant remains within a lake. Does this cast doubt on all of Warner's (e.g. Warner et al. 1984) careful plant macrofossil analyses?

While our focus has been on broad-scale processes, Schindler et al. (1990) have recognized the significant role that climate plays at a much finer temporal scale in regulating recent changes to aquatic systems. In the Experimental Lakes Area (Ontario), air and water temperatures have gradually increased during the past 20 yr and have been accompanied by declines in cold-stenothermous organisms, including those living beneath the thermocline (Schindler et al. 1990). Similar results have been reported for Lake Windermere, U.K. George and Harris' (1985) observations there suggest an intimate link between annual variations in climate and zooplankton biomass. Thus, Hann et al.'s contention that aquatic ecosystems are well buffered from climatic change, "probably within a temporal scale of 10^2 – 10^3 yr", is completely unsubstantiated. A further unsubstantiated and, in our view, incorrect claim by Hann et al. is that "Aquatic organisms thus respond more slowly to climatic change than terrestrial vegetation". Payette et al. (1989) studied the response of tree line to climatic change in northern Quebec. They concluded that "Although recent climatic data indicate sustained global warming during this century, no conclusive evidence of a positive vegetation response to such warming has yet been identified . . .". Payette et al. (1989) note, in fact, that "development of low-krummholz vegetation at these sites seems to be out of phase with the twentieth century warming".

Despite the similarities in the studies conducted by Payette et al. (1989) and Schindler et al. (1990), the aquatic ecosystem responded in a predictable manner; tree line vegetation did not. Similarly, Iversen (1954) presents a carefully argued analysis of the different ecological responses of terrestrial and aquatic organisms. His conclusions and those of very many other palaeoecologists are totally opposite to Hann et al.'s claim.

Davis (1984) states that "The most rapid responses to climate change occur among animals". The greater mobility of animals is a matter of common knowledge. In addition, Davis (1984) recognizes that forest species composition is very slow to change, the low rate of change being set by the long life span and low reproductive rates of forest trees. Modelling experiments by Davis and Botkin (1985) illustrate the inertial response of forests to climatic change.

The response of terrestrial vegetation and chironomids to late-glacial climatic changes can be directly compared at two sites studied in the Maritime Provinces, Splan Pond (Mott et al. 1986; Walker et al. 1991a), and Brier Island Bog (Wilson et al., unpubl. data). In both instances, the changes in chironomid species composition are much more abrupt and distinct than those changes depicted by fossil pollen from terrestrial taxa.

Hann et al. expend considerable energy outlining the importance of core versus marginal populations of organisms, but they fail to indicate its relevance to the questions under discussion. We refer them to Hengeveld's (1990) critical and pen-

etrating analysis on this important biogeographical and ecological topic and the possible mechanisms that control range dynamics at a range of scales in space and time.

Hann et al. state that our data set encompasses a broad range of lake types and that this somehow diminishes their utility in evaluating factors that control chironomid distribution. These lakes were actually selected to reduce the variability that exists among the tens of thousands of lakes in Labrador; they were selected so as to be of fairly similar size, and without drainage from upstream lakes or rivers. The geology of Labrador is not uniform, but for the most part is dominated by granitic Canadian Shield rock; the lakes are with few exceptions dilute, oligotrophic, unperturbed systems. Of course there is limnological variation among the study sites, but these differences do not obscure the overriding influence of latitudinal temperature on benthic communities.

Ideally, we would like to have collected much more extensive temperature data for our sites, and Hann et al. have criticized us for this deficiency. The distributions of chironomids might be regulated by maximum summer water temperature, annual degree-days, etc., but expecting us to have these data for all sites is unrealistic. The expense of stationing a person, or a data-logger, on every one of these remote lakes for several years would be prohibitive.

Although the Labrador lakes were sampled over about a 1-mo period, the southeastern sites (Nos. 1–20) were visited earlier (July 22–26) than the northern sites (Nos. 21–66; August 18–23). What this means is that the lakes south of Goose Bay were sampled during one brief time-window and all those to the north were sampled during another equally short period. Because the northern lakes were sampled later in the summer, their temperature should have been somewhat higher than had they been sampled in late July (summer comes late to the tundra). Thus, if there were any bias in the data set, it would be one which would shrink the latitudinal temperature gradient. That our single measurement of temperature is still highly correlated with latitude underscores the large north–south thermal gradient in Labrador lakes and its importance in controlling chironomid distributions.

Hann et al. point out that hypolimnetic temperature influences the benthic fauna, but that we reported only lake surface temperatures. However, few of the lakes in the Labrador training set showed any serious thermal stratification at the time of sampling (regrettably not mentioned in Walker et al. 1991b). In fact, all but three were effectively isothermal (1–2°C difference top to bottom), either because of wind stress (tundra and forest–tundra sites) or shallow depth (many in the southeast). Only a single lake, amongst all of those used in our statistical analyses, was thermally stratified. Therefore, benthic communities in these lakes (and very many others) are not isolated from climate.

We initially ran the statistical analysis without using the temperature data; latitude and depth together explained almost as much of the variance of the weighted averages of taxa as do temperature and depth. Because latitude and all temperature variables in Labrador will be highly correlated, it makes little difference to our conclusions how we express the thermal gradient; there is a distinct change in chironomid faunal composition with changing latitude and depth. This relationship is dramatically portrayed in fig. 5 of our paper.

As Birks et al. (1990, p. 264) have stated, one of the basic assumptions of all transfer function work in quantitative palaeoecology is that “The environmental variable to be reconstructed is, or is linearly related to, an ecologically important variable

in the system of interest”. Shuter et al. (1983) demonstrated that strong correlations among thermal variables do exist; thus, since chironomid distributions are correlated with summer surface water temperature, it is not unreasonable to reconstruct temperatures on the basis of the chironomid data.

Hann et al. have raised concerns about our statistics because they consider them to be “complex” and “questionable”. We make no apology for using complex statistics to address a complex problem, nor for using the most appropriate statistical tests currently available! Unless Hann et al. can clearly specify why they consider the statistics “questionable”, we cannot address their concerns. Canonical correspondence analysis (CCA) and weighted-averaging calibration are being used routinely in ecology, palaeolimnology, and palaeoecology (e.g. Birks et al. 1990; Fritz et al. 1991; Kingston et al. 1992; ter Braak and van Dam 1989; Walker et al. 1991a), and over 120 papers using CCA have been published in the peer-reviewed literature since 1986 without substantive questions about this method being raised.

We are disturbed by Hann et al.’s disregard for tests of hypotheses constructed a priori versus a posteriori. Our conclusions regarding the strong influence of temperature and depth, and irrelevance of sediment organic content, are directly the result of statistical testing via partial CCA (ter Braak 1988). These tests addressed hypotheses that had been stated by Walker and Mathewes (1989b) and Warwick (1980, 1989), long before our study (Walker et al. 1991b) was initiated. They cannot be treated on the same basis as the results of the exploratory CCA, in which no a priori hypotheses are stated. The claim of Hann et al. that our null hypotheses are “all inadequately tested” implies that partial CCA (ter Braak 1988) with its battery of Monte Carlo permutation tests (ter Braak 1990) is inadequate statistically. The great power of hypothesis testing with partial CCA was demonstrated, for example, by Pysek and Leps (1991).

It appears that Hann et al. may have misunderstood our statistical treatments by suggesting that values in our text and table 2 (Walker et al. 1991b) do not correspond. The value in the table represents the variance (i.e. 0.20) accounted for by temperature. The total variance explainable, with all environmental variables included, is 0.91; thus, the proportion of total variance explained by temperature is 0.20/0.91 (= 22%), as we stated (Walker et al. 1991b, p. 978).

In reference to our comparison of inferred versus predicted Labrador water temperatures, Hann et al. accuse us of having “been snared in the trap of circular reasoning”. This is completely unacceptable because we clearly indicated the deficiencies of the comparison and stated (Walker et al. 1991b, p. 980) that our model “is much more rigorously tested” with an independent test set. The test of our model is the British Columbia results, not the Labrador training set. To state as Hann et al. do, that “transfer functions should be validated using *independent modern data sets* and not the *same data set used to develop the model initially*” implies that we did not do this! Our paper is one of the very few on transfer functions that actually does what Hann et al. say, quite correctly, should be done (see Birks and Gordon 1985; ter Braak and van Dam 1989). Why therefore do Hann et al. not accept the British Columbia data as a rigorous test? The fit of r^2 of 0.53 ($r = 0.73$, prob. < 0.001) is not, in a statistical sense, a “poor fit”. What additional criteria of goodness-of-fit do they propose applying?

Hann et al. insist that we should make some sort of arbitrary separation between littoral and profundal chironomids. Not only is this idea unworkable, but we would be discarding much of

the data on which the models are based. Our paper includes a more insightful analysis of the model's capabilities and clearly specifies the constraints to be used in its application. Any taxon that is distributed without regard to climate has little or no influence on the coefficients used by the model for temperature inferences. By excluding the profundal taxa, the model would be less reliable. Further, as has been shown with other limnetic indicators (e.g. diatoms), it is advantageous to integrate the communities spatially. A lake-centre sample is likely to contain as many taxa as possible from the various habitats within the lake.

In addition, our analysis suggests that independent reconstructions of temperature and depth are possible. This is particularly exciting because both variables have considerable palaeoclimatological significance — contrary to the view of Hann et al. As Harrison and Metcalfe (1985) indicate, pronounced fluctuations in lake depth may have occurred in response to changes in evaporation/precipitation balance; thus, lake-level changes may be key indicators of past precipitation regimes. Kingston et al. (1992) demonstrate that statistically independent reconstructions of several environmental variables are possible from palaeoecological data. Such methods are revolutionizing quantitative palaeoecology.

Hann et al. state (1) "there is considerable risk involved in extrapolating from one region to another in North America let alone extrapolating from a European ecological database", and (2) "Climatic inferences based upon species presence/absence in an assemblage also need to be interpreted very carefully". We made use of no European data in our analysis, and our CCA and weighted-averaging techniques are not based on presence/absence data.

In conclusion, we had hoped that the data presented in Walker et al. (1991b) would have contributed towards the resolution of this long-standing and rather sterile debate. It is our hope that, as data continue to amass supporting our hypotheses (e.g. Rossaro 1991; Walker 1991; Walker and Mathewes 1989b; Walker et al. 1991a; Wilson et al., unpubl. data), we will be able to focus our energies on more productive challenges, such as the exciting, urgent, and important problems being posed by global change to palaeoecologists worldwide. Investigation of such questions requires careful project design, sound taxonomy and good ecological knowledge, extensive training sets, independent test sets, carefully collected fossil data, and appropriate multivariate statistics. Quantitative palaeolimnology is now well able to answer many of the key questions raised in global climate change research — Ian R. Walker, *Department of Biology, Okanagan College, Kelowna, B.C. V1Y 4X8, Canada*, John P. Smol, *Department of Biology, Queen's University, Kingston, Ont. K7L 3N6, Canada*, Daniel R. Engstrom, *Limnological Research Center, University of Minnesota, Minneapolis, MN 55455, USA*, and H. J. B. Birks, *Botanical Institute, University of Bergen, Allégaten 41, N-5007 Bergen, Norway*. (JB306)

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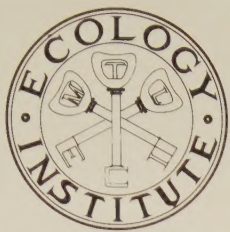
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